Haematological and nutritional status of Sudanese women with sickle cell trait and anaemia: Does sickle cell trait compromise birth outcomes?

A thesis submitted to London Metropolitan University in fulfilment of the Degree of Doctor of Philosophy

By

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In the name of God, the Kind, the Caring

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Dedication

To my wife, children and family, who gave support and encouragement.

To my friend: Mohammed Eltyeeb, a generous and supportive figure.

Author's declaration

I certify that the thesis titled: **Haematological and nutritional status of Sudanese women with sickle cell trait and anaemia: does sickle cell trait compromise birth outcome?**

Which is submitted for the degree of Doctor of Philosophy has not been previously submitted for another degree in this or any other educational institution. Moreover, I confirm that the clinical research, laboratory analysis, data analysis and interpretation of the data are entirely my work, except where otherwise acknowledged in the main text of the thesis.

Name: Signed… Eltigani Hassan Ali …

Date……01/6/2022……

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Acknowledgements

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Abstract

This research programme has investigated the haematological and nutritional status of Sudanese women who have both sickle cell trait and sickle cell anaemia and has considered whether women with sickle cell trait experience worse outcomes of childbirth than those without.

Non pregnant group with sickle cell anaemia:

In this study, (n=39) sickle cell anaemia and healthy control (n=36) non-pregnant childbearing age women were recruited, anthropometric data and blood samples were collected for haematological study. The aim was to study the effect of sickle cell anaemia on the general health and nutritional status of Sudanese women.

Sickle cell anaemia affects the nutritional status of women of childbearing age. The women who were involved in this study were noted to be shorter than the healthy women, and they had low body mass indices and lower weight than the average for their unaffected peers. Consanguineous marriage is more prevalent among people with sickle cell anaemia than among the wider population. Sickle cell anaemia affects the lifestyles of women of childbearing age and on average they attain lower educational qualifications than those who do not have the disease. In our previous study 2016, we found that sickle cell prevalence was high at 24.9% in the west of Sudan.

Pregnant sickle cell trait group:

Then as there was no registry for sickle cell trait in pregnant women we have to do some screening in the antenatal clinic to select our study group.

At their first antenatal visit, 367 pregnant women were screened for sickle cell trait through the use of haemoglobin electrophoresis. Of these, 34 were diagnosed with the condition. Pregnant women with sickle cell trait (HbAS) were found to exhibit abnormal haematological measurements late in pregnancy, and these measurements were lower than those in a group of healthy pregnant Sudanese women who comprised a control group. Women with sickle cell trait carried increased risks of miscarriage and stillbirth, and their babies weighed less at birth than those born to healthy women with normal haemoglobin (HbAA).

Fatty acids levels in sickle cell trait pregnant women.

Unexpectedly, pregnant women with sickle cell trait were found to have higher levels of fatty acids, in both the plasma and red blood cells (RBCs), than the control group, both early and late in pregnancy. The women with HbAS compared with the women without, had higher levels of saturated and monounsaturated fatty acids both early in pregnancy and late, until the time of delivery. However, in this study, it was found that healthy Sudanese pregnant women, with no sickle cell trait, had lower levels of fatty acids than pregnant women in other countries.

Conclusion

This pilot study indicates that sickle cell anaemia affects the general health of Sudanese women, Pregnant Sudanese women with sickle cell trait face many challenges during pregnancy and childbirth, and they require more support than they receive currently. Health education and screening programmes should be implemented. Children born to women with sickle cell trait must be evaluated at birth and closely followed up to discover whether they carry the sickle cell gene and to evaluate their fatty acid levels to improve their nutritional status. Provision of fatty acid supplements for pregnant women with sickle cell trait during pregnancy and lactation should be considered. There is a need for further studies to increase understanding of the

biological situation among pregnant women who have sickle cell disease and to improve their childbirth outcomes.

Publications

Peer-reviewed and Conference Publications

Peer-reviewed publications

1. **Ali, E.H,** Salam Alkindi, Mohamed A. Osman, Wafa Hilali, Hind M. Mirgani, Gareeba Adam, Magdi M Morsi, Izzeldin S Hussein and Kebreab Ghebremeskel (2021) **Nutritional and Haematological Status of Sudanese women of Childbearing Age with Steady-state Sickle Cell Anaemia.** *Oman Medical Journal 36(3):e270*.
2. **Ali, E. H**., Alkindi, S., Mohamed, A. O., Awadalla., K.E, Abdlgadir, O., Adam, G., Magdi M., Ibrahim, A. K., Ghebremeskel K (2022) **Adverse pregnancy outcomes in patients with Sickle cell trait-prospective cohort study: Sickle cell trait pregnancy and outcomes.** *Mediterranean Journal of Hematology & Infectious Disea*ses (MJHID).
3. **Ali, E.H** (2021) **Blood Fatty Acids of Sudanese Pregnant Women with Sickle Cell Trait.** *Journal of the American Oil Chemists Society 98, 241-242.*

Conference publications

1. **Ali, EH,** Abuknesha N, Ghebremeskel K (2021) on **Blood fatty acids of Sudanese pregnant women with sickle cell trait. 1**4th Meeting of the International Society for the Study of Fatty Acids and Lipids (ISSFAL), on line poster presentation.
2. **Ali, EH (**2018) **Pregnancy and birth outcomes and essential nutrient status of Sudanese pregnant women with sickle cell disease and sickle cell trait.** International Symposium on Sickle cell disease symposium - current trends of prevention and treatment. Khartoum, Sudan. Sudan medical speciality Board.

   Organiser and speaker.

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Abbreviations

AA	Arachidonic acid
Bp	Blood pressure
BHT	Butylated hydroxytoluene
BMI	Body Mass Index
CAR	Central African Republic
DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EPA	Eicosapentaenoic acid
FAME	Fatty acid methyl ester
ESRD	End-stage renal disease
FAO	Food and Agriculture Organisation
FID	Flame ionisation detector
FMOH	Federal Ministry of Health
G6pd	Glucose 6-phosphate dehydrogenase
GAG	Guanine-adenine-guanine (genetic code)
GLC	Gas-liquid chromatography
GTG	Guanine-thymine-guanine (genetic code)
Hb	Haemoglobin
HbAA	Healthy adult haemoglobin

HbAS	Haemoglobin in sickle cell carrier state A/S (sickle cell trait)
HbC	Haemoglobin C
HbD	Haemoglobin D
HbF	Haemoglobin F
HbSS	Homozygous sickle cell disease
HPLC	High-performance liquid chromatography
HSCT	Haematopoietic stem cell transplantation
ICU	Intensive care unit
IDA	Iron deficiency anaemia
KAP	Knowledge, attitude and practice
KSA	Kingdom of Saudi Arabia
LA	Linoleic acid
LLE	Liquid-liquid extraction
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
MOH	Ministry of Health
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
NO	Nitrous oxide
NaCl	Sodium chloride (Normal saline)

OFN	Oxygen-free nitrogen
PCV	Packed cell volume
PF	Plasmodium falciparum
PLTs	Platelets
PUFA	Polyunsaturated fatty acid
RBC	Red blood cell
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SCBU	Special Care Baby Unit
SCA	Sickle cell anaemia
SCD	Sickle cell disease
SCT	Sickle cell trait
SD	Standard deviation
SPSS	Statistical package for social science
TWBC	Total white blood cell count
UN	United Nations
UNICEF	United Nations International Children's Emergency Fund
VD	Van deemer
VLA-4	Very late antigen -4
VOC	Vaso-occlusive crises
WHO	World Health Organisation

ZPP/H	Zinc protoporphyrin /haemoglobin
α	Alpha
β	Beta
γ	Gamma
ζ	Zeta
€	Epsilon

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Chapter 1

1. Introduction and literature review in sickle cell disease

1.1 Introduction

Sudan is a country located in the northeast of the continent of Africa. It is bordered in the north by Egypt, in the east by Eritrea and Ethiopia, in the south by South Sudan and in the west by the Central African Republic (CAR), Chad and Libya.

The White Nile comes from the south and joins the Blue Nile which is coming from the east at Khartoum, in the centre of the country, to form the Great River Nile. After South Sudan gained independence in 2011, North Sudan (Sudan) now measures approximately 2,500,000 square kilometres (UNFOA, 2013).

The population is estimated to be 37.9 million people, most of whom are young, and the mortality rate among those under five years old was 83 per 1000 live births in 2010. The most recent study found that the maternal mortality ratio was 216 women per 100,000 live births (World Health Organisation (WHO), 2017). The country has been exposed to a great deal of conflict and instability, which has disrupted the health system and economy. Population growth has exceeded the expansion of the health sector so that established facilities no longer have the necessary staff and skills. Only 60% of women receive antenatal care, maternal mortality is on the rise, and preventive and curative health services are in very limited supply (United Nations (UN), 2004). Women from North Kordofan, an arid and desert region in the centre of the country, show a high prevalence of chronic energy deficiency of up to 32%, according to the Sudanese Ministry of Health (MOH) and the WHO (MOH/WHO, 1997).

The Federal Ministry of Health (FMOH) developed a strategic plan to improve health services and ensure livestock production as a source of food. To implement this plan and to ensure its success, it has been stated that rural to urban migration should be reduced and displaced people settled (Fadlalla and Ahmed, 2003).

Due to war, tribal fights and instability, most families have migrated to the big cities in search of education, better health facilities and peace.

The health sector is weak due to the economic situation, which deteriorated after the separation of South Sudan and the consequent loss of oil revenue, as well as a trade embargo and sanctions that have been imposed on the country over the last three decades. The government has had a policy of free health care since 1995, yet population growth and economic instability have hindered the achievement of this goal. Free emergency care and health services for children under five years old have been implemented since 2006/2008, but again, these policies have not been widely adopted (Gaafar and Reem, 2014).

In Sudan, health services are provided by the FMOH, military medical centres, the private sector and a few other institutions. The FMOH provides health care at three levels within district localities: primary, secondary and tertiary (Muhammad, 2017).

Health care services offer vaccinations and nutrition, reproductive health and preventive-care services. Inherited and genetic diseases are investigated and treated only at the tertiary level. In rural areas, most of these diseases are treated by native healers and/or herbal medicine.

One of these genetic blood disorders is sickle cell disease (SCD). The inheritance pattern of this disease is autosomal recessive (Sabahelzain, 2014). It is the second most common cause of anaemia in children and adults in Sudan. Because health education programmes do not reach

remote areas, many people have poor knowledge and outdated opinions of this disease, even though it is commonly found in some tribes.

In my practice, I notice that people with sickle cell trait (SCT) may live until old age despite their status as carriers of mutated genes. Pregnant women with SCT are generally not diagnosed and are treated in general antenatal clinics, in which no treatment is targeted at their genetic condition. In most cases, they do not show any serious complications unless they are exposed to stressful situations, such as blood loss, infection or toxaemia late in pregnancy.

Because they are not diagnosed they are treated and given symptomatic treatment for pain, giddiness or even anaemia without consideration of an SCD diagnosis.

Members of many tribes in the rural areas of Sudan marry at young ages. Arranged marriages are common in the west of Sudan, such as in Kordofan Province, leading to a high frequency of cousin marriages. Such a high frequency of consanguineous marriage can result in a high prevalence of autosomal recessive genetic diseases such as SCD (Mansour *et al*., 2011). Most of those who are affected do not know they are carriers until they develop symptoms of SCD or participate in screening programmes. Carriers face no impediment to marriage because, under normal circumstances, they do not display symptoms.

The burden of sickle cell disease (SCD) is increasing. A planned national policy in the public health sector is required to reduce the effect of SCD on the health of women and their offspring (Piel *et al.,* 2013.).

1.2 Background

The area chosen for this study was Kordofan Province, in the west of the country (Figure 1.1). El-Obeid is the capital of the province. The population is around 1.5 million. The people who

live in El-Obeid are a mixture of farmers and nomads who have settled in the city and make their living in trade or local services. Some have become government employees.

The climate is savannah-like; a dry season from February to April is followed by a rainy season from May to October and a short winter season that lasts only from November to January.

1.2.1 Status of health in Kordofan Province

Reasonably good medical services are more common in the cities than in rural areas. Besides the central government hospital, the military hospital and the police hospital, there are around nine small health centres scattered throughout the city, but they lack adequate staff and medical equipment, as well as facilities for simple investigations and common treatment.

As in other African countries, the Sudanese FMOH tries to control the impact of SCD (Ali *et al*., 2012) with limited diagnostic and management facilities. However, these efforts are hindered by poor funding, as the limited health budget is directed toward other diseases.

There is only one haemoglobin electrophoresis machine, which can be used to diagnose SCD, to serve the entire province and neighbouring areas. Reagents are not available most of the time; when they are ordered, the delivery may take two months. The machine requires maintenance and technicians have to come from Khartoum. I have been able to overcome these challenges, albeit at a financial cost.

Kordofan local government has provided some support to set up a Sickle Cell Disease Centre in El-Obeid. The centre is composed of three rooms: a clinic, an office for the centre director and a laboratory and equipment room. The centre still requires more funding for equipment, trained staff and creating a budget specifically for reagents and consumables.

Patients with SCD now have access to health insurance coverage for doctor visits, investigations and treatment. The centre holds one clinic per week to serve all the registered

patients, which currently number about 1,800 people. Patients must have appointments to attend the clinic.

Figure 1-1 Map of Sudan (World Atlas, 2014)

1.3 Literature review

History and origin of sickle cell disease

SCD is an inherited blood disorder. It is transmitted as an autosomal recessive disease that creates characteristic changes in the sickle haemoglobin (HbS) gene, which results in chronic haemolytic crises and inflammation. SCD is a major cause of morbidity and mortality worldwide. In West Africa and among the black population of the USA, the mortality rate has reached 11.5% (Sun *et al.,* 2001), but with improved health services, that figure has decreased to 1% (Ashish *et al.,* 2008). In SCD, mortality is sometimes related to the development of pulmonary hypertension and increased cardiac output that is caused by anaemia (Mehari *et al.,* 2013).

SCA is caused by the homozygous form of the HbS gene (HbSS) and SCT is a heterozygous state sickle cell trait (HbAS) (SCT). There are other combinations and variants such as sickle cell β-thalassaemia and sickle cell with an associated deficiency in the enzyme glucose-6-phosphate dehydrogenase.

Mothers with SCT or fathers who carry the mutated gene are the most likely sources of transmission to the next generation. Stroke survivors with SCT tend to have poor prognoses (Olowoyo et al., 2016). Pregnant women with SCA contribute to transmission.

A person with SCT does not suffer any symptoms under normal circumstances, and it is undetectable in routine tests for illness. The detection can be specifically by taking sample of blood and do haemoglobin electrophoresis.

Earl (1970) reported the case of a person with SCT who developed severe pneumonia and died at the age of 35 years. During intensive exercise, some athletes have experienced sudden death due to SCT, according to Harris *et al*. (2012).

Carriers of SCT tend to have poor prognoses following strokes (Olowoyo *et al*., 2016). Cancer patients also have an increased risk of poor outcomes if they carry the gene for SCT (Swede *et al*., 2015). Transmission of the disease to the next generation is mostly through mothers who have SCT or fathers who are carriers of the mutated gene. In developing countries, very few men who have SCA live to marry and transfer the disease.

SCA and SCT individuals show symptoms when they are exposed to stress, infection or dehydration. These stressors cause them to develop vaso-occlusive crises, which then occur frequently. These crises are characterised by severe pain due to ischaemia of the tissues, which can lead to organ damage. In developing countries, the mortality rate due to this condition is increased due to the lack of health facilities. Most patients are not aware of these complications and possible outcomes and tend to use local native treatments to manage pain or to treat jaundice. They seek medical treatment only at the very late stages of the disease. In some tribes in Africa, people hide the disease because they think it is infectious (*based on personal communication with Mrs Huaida, Sudan, 2014).* In the west of Sudan, some women avoid visiting a woman who delivers a baby at home if she is known to have SCT or SCA, as they believe that she is infectious (*personal communication with Mrs Huaida, Sudan 2014*).

In western countries where health services are advanced and available, sickle cell patients are monitored from birth. The disease can be diagnosed in the foetus antenatally and all precautionary measures are followed during pregnancy. After delivery, the at-risk child receives the necessary treatments and vaccinations, so that complications may be delayed. Issues that occur can be minimised and managed through regular follow-up.

1.3.1 Sickle haemoglobinopathy

Investigators have discovered many haemoglobin variants so far. Of these variants, haemoglobin S (HbSS) is the most common. It occurs when glutamic acid is replaced by the

neutral amino acid valine at the sixth position in the β-chain of haemoglobin. Haemoglobin C (HbC) is formed when glutamic acid is replaced by lysine. The mutation may show up in the form of HbSS or a compound heterozygote is formed with another haemoglobin variant (Oteng-Ntim *et al.,* 2008; Rajab and Sherman, 2004*).*

Haemoglobin is understood to comprise four polypeptide chains, each with an attached haem molecule. The haem consists of an iron molecule attached to four pyrrole rings. Two pairs of globin chains (two α and two β) attach to the pyrrole rings to form haemoglobin (Oteng-Ntim *et al.,* 2008; Rajab and Sherman, 2004).

1.3.2 Haemoglobin structure and fundamental factors

At the early embryonic stage of baby development, haemoglobin is composed of two zeta (ζ) and two epsilon (€) chains, which form what is called Hb Gower I, and then two alpha (α) and two € chains form Hb Gower II. However, during the foetal stage, haemoglobin alters so that the main form is so-called foetal haemoglobin (HbF), which is composed of two alpha and two gamma (2α, 2γ) chains. Typically, after the child reaches six months of age and into adulthood, most of the haemoglobin (up to 96%) becomes HbA, which is composed of two α and two beta (β) chains. The alpha chain contains 141 amino acids and the β chain contains 146 amino acids (Serjeant and Serjeant, 2001)(Sickle Cell Disease 3rd edition).

Haemoglobin synthesis can be explained by the study of genetic information in terms of protein structure in the cell nucleus of deoxyribonucleic acid (DNA) molecules. DNA is a double-stranded, helical molecule, each strand of which is composed of alternating phosphate and deoxyribose molecules. Also attached are other major bases, purine, adenine and guanine, and the pyrimidines cytosine and thymine. Both ends of DNA have carbon atoms (5’ at one end and 3’ at the other). The process of haemoglobin synthesis is complex and ultimately balanced.

This complex process requires the globin chain to be synthesized in the ribosomes, where mRNA is read and translated (Serjeant and Serjeant, 2001).

1.3.3 Molecular basis of SCD

In HbSS, a single-point mutation occurs in which the codon determines the amino acid at position β6. Usually, the codon for glutamic acid is guanine, adenine, and guanine (GAG), but the mutation converts it to guanine, thymine, guanine (GTG), which is the codon of the valine amino acid. Several other blood disorders, such as thalassaemia, HbO-Arab, HbC, HbD, and HbD Punjab, have different pathogenesis, but SCD can result from HbS inheritance in combination with any of these types. In some areas, this can result in severe illness. (Hoffbrand and Pettit, 1992). In my clinic I see one patient with sickle cell anaemia and thalassaemia, and another patient with sickle cell anaemia and g6pd in one family.

Table 1-1 Molecular pathophysiology of SCA (Hoffbrand and Pettit, 1992)

Amino acid	Proline	glutamic acid	glutamic acid
Normal β chain Base composition[	CCT	GAG	GAG
Base composition	CCT	GTG	GAG
Sickle β chain Amino acid	Proline	valine	glutamic acid

A = adenine, C = cytosine, G -=guanine, T = thymine

Although SCD is inherited as an autosomal recessive trait that can present in mild form as heterozygous when one parent carries the mutant gene, it can be considered dominant in the sense that HbS is always expressed in those who inherit the gene from either parent. It is also

considered codominant as it is expressed in the heterozygous condition (Serjeant and Serjeant, 2001).

Serjeant and Serjeant (2001) provide highly useful information by application of the Hardy-Weinberg principle, which shows how inheritance of the sickle cell gene proceeds in each generation. Genotype frequency remains constant according to the formula

$$p2 + p\,q + q2 = 1,$$

in which p and q represent the frequencies of each genotype. Some factors may limit the application, such as selection, non-random mating, mutation, drift and gene flow (Serjeant and Serjeant, 2001).

1.3.4 Discovery of SCD and distribution

Edelstein (1981) reports that SCA was discovered in Ghana in 1670. Physician James Herrick was the first to report the disease at a molecular level when in 1910 he reported observations of elongated, sickle-shaped RBCs in the peripheral blood film of a dental student from Grenada.

Emmel (1917) concluded that, on a genetic basis, the inheritance could be a Mendelian autosomal recessive type. Huck and Sydenstricker (1923) studied numerous cases and proved the genetic background of SCA. Hahn and Gillespie *(*1927) proved in their studies that the sickling phenomenon was explained by exposure to low oxygen tension (hypoxia).

1.3.5 Inheritance of the sickle cell gene

The genetics of the sickle cell was studied by many researchers and were found to follow the Mendelian recessive pattern. For children of one carrier parent who had sickle cell trait (HbAS) and one non-carrier parent, there would be a 1:2 chance that they would be a carrier of HbS, regardless of whether they represented the mother's first or second pregnancy (Serjeant and Serjeant, 2001).

Therefore each child has a 50% chance that they will have sickle cell trait (HbAS) or they will be non-carriers (HbAA).

Figure 1-2 Inheritance pattern of the sickle cell gene.

http://www.idph.state.il.us/HealthWellness/sicklecell.html

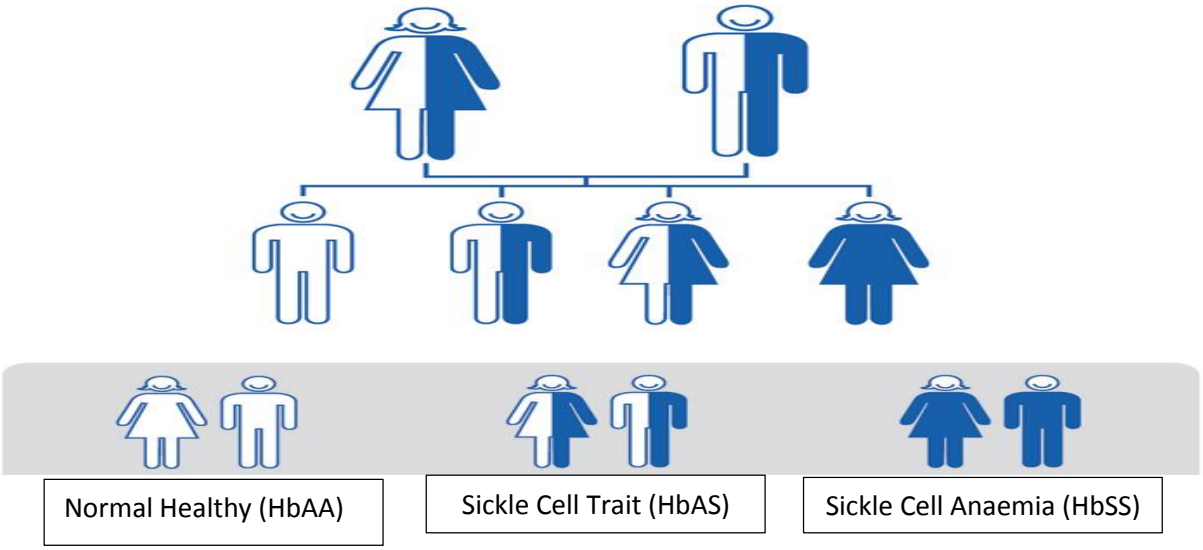


Figure 1-3 Inheritances if both parents were SCT

Adapted From (http://www.idph.state.il.us/HealthWellness/sicklecell.html)

If both parents have SCT (HbAS), each child has a 50% chance that they will be carriers (HbAS) a 25% chance that they will be non-carriers (HbAA) and a 25% chance that they will have sickle cell Anaemia (HbSS). A genetic counsellor may help to educate parents who carry the gene at the time of diagnosis.

The discovery of haemoglobin electrophoresis affected a great change in the knowledge of SCD (Beet, 1949; Neel and Itano, 1951). Some official reports suggest that about 20 million to 25 million people are homozygous: of these, 12 million to 15 million are in sub-Saharan Africa, five million to 10 million in India and nearly three million in other parts of the world (Serjeant, 2006). The WHO reports that about 300,000 infants are born with the disease each year, two-thirds of whom are born in Africa (WHO, 2006). The frequency of occurrence of SCT (HbAS) is high in the Kingdom of Saudi Arabia (KSA), Greece, India and Africa, ranging between 25% and 40% (Serjeant and Serjeant, 2001).

Screening of new-borns who are at high risk of developing SCD is a common practice in the USA and some European countries since it enables clinicians to detect SCD complications in adults and can help patients to live longer than they might if they are not diagnosed.

In Africa and the Middle East, screening is not yet well established and most babies are born at home (Ryan *et al.*, 2010), sometimes among nomadic peoples, in the jungle or under a tree. Therefore, their genetic condition is undetected. Some young patients are not treated until they experience growth failure or other complications such as hip necrosis.

1.3.6 Sickle cell gene mutation and epidemiology

According to Lehmann (1954), the origin of the disease can be explained according to the single-gene mutation theory. This theory suggests that the disease first appeared on the Arabian peninsula long ago when the climate changed from rainy to dry, and the previously green area

became desert. The single-gene mutation had been confined to residents of the peninsula, but when the change of climate forced people to move, those with SCD and SCT moved to Africa and India. As the migrants passed the abnormal gene to their children and the migration occurred in different directions, the mutation became widespread (Lehmann, 1954) (Figure 1.4).

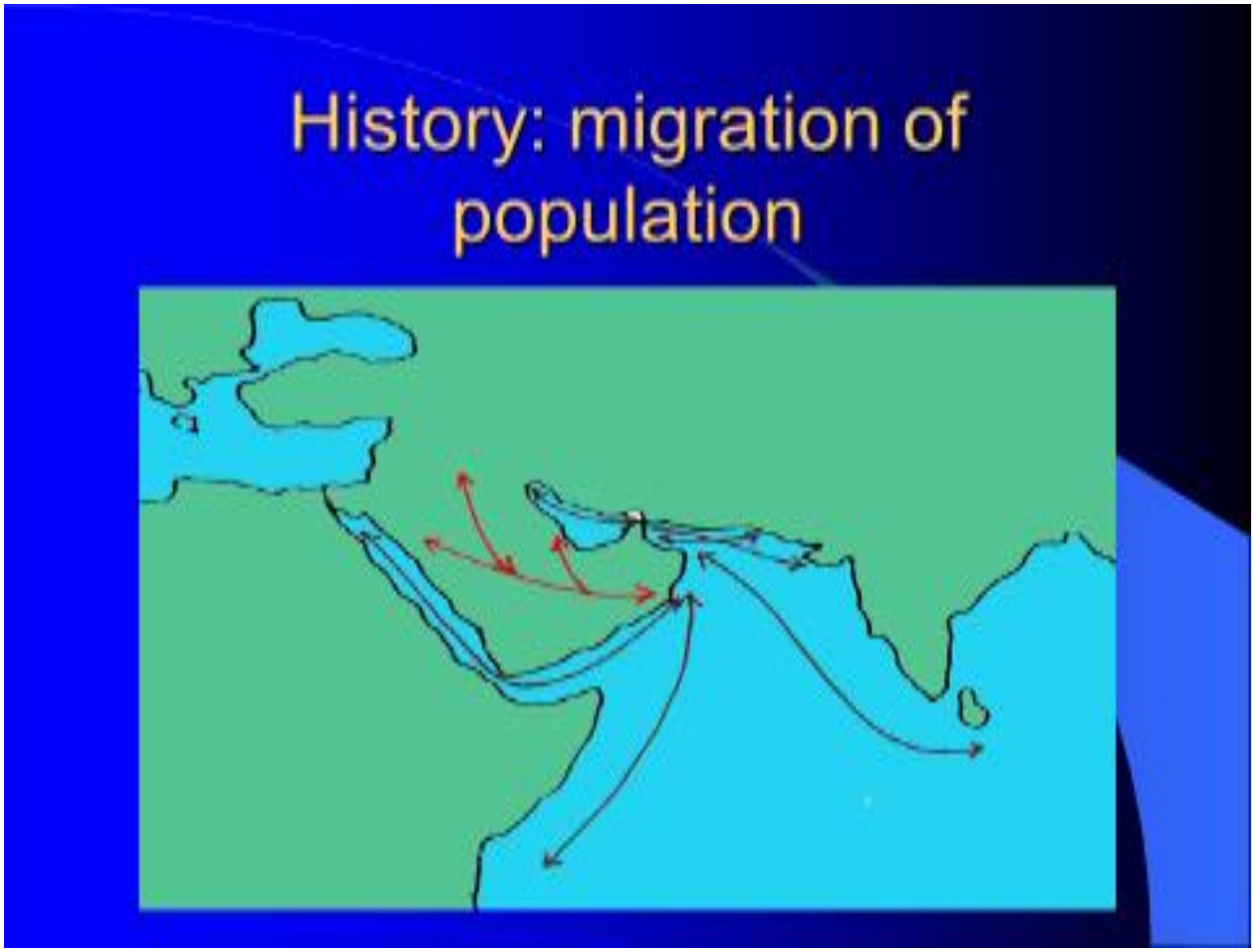


Figure 1-4 Migration of people from the Arabian Peninsula to other areas led to the spread of the single-gene mutation that causes SCT and SCD

(reprinted courtesy of Prof. S. Alkindy, Department of Haematology, Sultan Qaboos University Hospital, Oman).

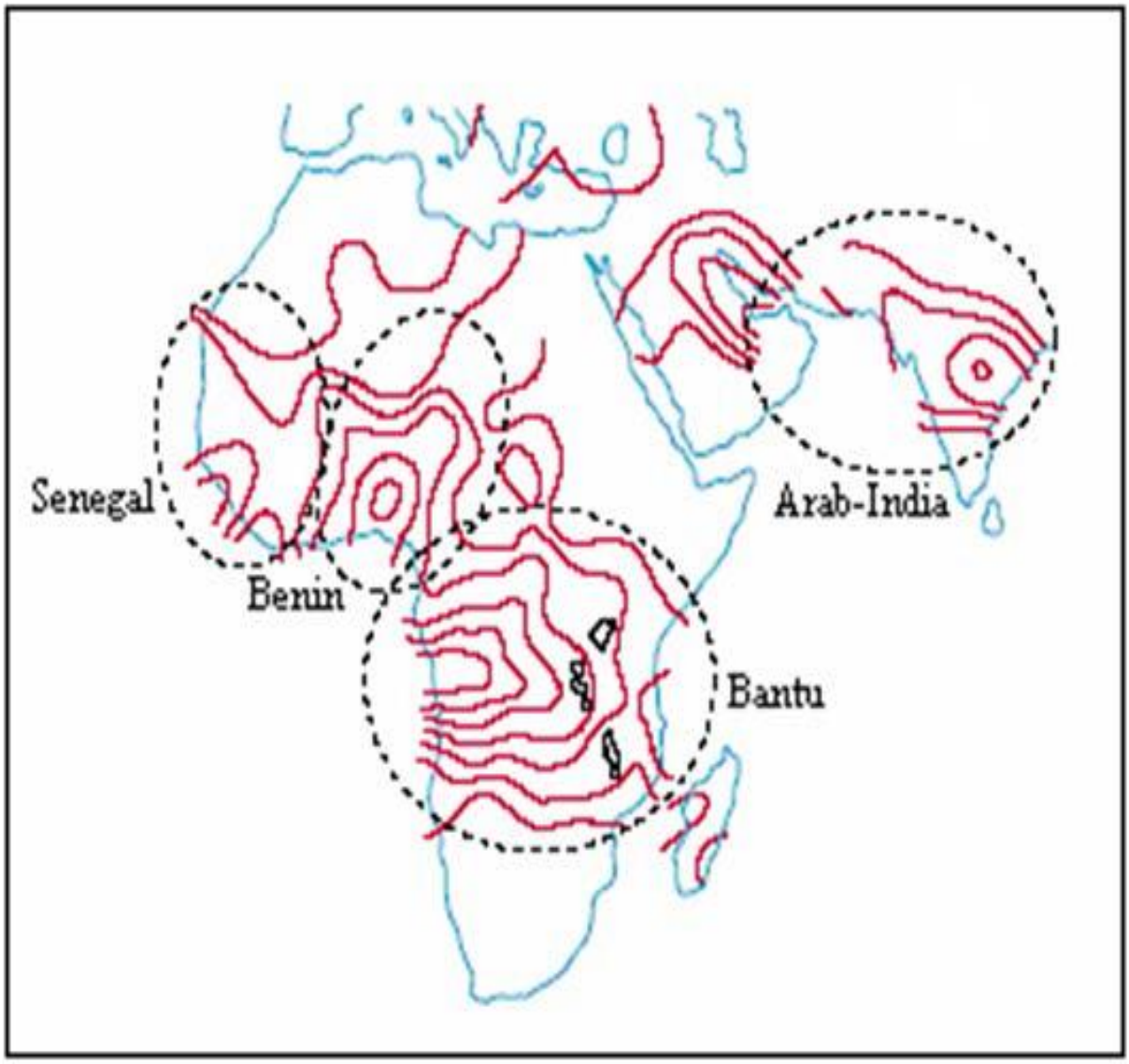


Figure 1-5 Map identifying the three distinct areas in Africa and one in the Arab-India region where the sickle gene is present (dotted lines) and individuals with sickle cell disease (red lines). (Source: Stuart, 2004).

The HbS gene in Africa is known to be associated with three haplotypes that resulted in mutations found in western and central Africa. These haplotypes are the Benin haplotype in Nigeria, the Senegal haplotype in West Africa and the Bantu haplotype, which occurs in the CAR and countries in the south of central Africa. A fourth haplotype is found in the KSA, particularly in the east, and in India (Lehmann, 1954) (Figure 1.5). Some African haplotype

genes are also found in western India. In the past, some Africans moved to India and settled there (Lehmann, 1954). People who were taken as slaves from Africa to North and South America carried the gene with them in the form of SCT or specific Benin, Senegal or Bantu gene haplotypes (Steinberg *et al.*, 1995).

In India, the Asian haplotype gene for SCT has reached up to 30% occurrence in some tribes. Beyond India, the disease was not known to spread among the general population, but rather only among emigrants (Serjeant, 1974). In these areas, other haematological diseases such as thalassaemia and glucose 6-phosphate dehydrogenase deficiency were more prevalent.

The sickle mutation haplotypes have different clinical presentations and different degrees of severity in the USA. The Bantu or CAR haplotype is the most severe, Benin is moderately severe and the Indian and Senegal haplotypes are of minor severity. It has been observed that a high level of HbF is apparent in the mild Senegal haplotype (Steinberg *et al*., 1995).

Multiple mutation theories and DNA polymorphism studies have been used to explain that the origin of the disease was equatorial Africa. Surveys that were conducted in the 1950s among older children and adults show a strikingly low prevalence of homozygotes (Allison, 1956; Lehmann and Raper, 1956), and these initiated the concept of balanced polymorphism. It was thought that heterozygous people were more fertile than homozygous. However, it has been shown that fertility has nothing to do with mutation, as there is no observable difference between males with SCT and males with normal HbAA in terms of gonadal function, testicular volume, hormones or the mean serum concentration of testosterone and pituitary function (Ezeh, 1996).

Another widely accepted theory was that these people had some resistance to malaria because they lived in an environment where malaria was endemic (Raper, 1949).

1.3.7.1 Mediterranean region

Sickle cell genes have been found in this region, in Italy, Greece and part of Turkey. People originally affected by the disease were of Caucasian origin with light-coloured skin and blue eyes. The genes were of the Benin haplotype, transmitted from Africa across northern African countries either through trade or by slaves taken by the Arabs, Franks and Ottoman Turks between the 10th and 18th centuries (Nagel, 1984).

1.3.7.2 Europe

The sickle cell gene was initially brought to the UK from the Caribbean, but this introduction was later considered to be due to immigration from West Africa, Ghana, Nigeria and Central Africa. In France, it originated from the North African population who settled there. The same applies to Germany, where the gene originated with Turkish immigrants who acquired it from African slaves (Nagel *et al,* 1984).

1.3.7.3 USA and the Caribbean

The disease was introduced to this area during the period 1650 to 1830 due to the slave trade. It originated in West Africa. The SCT gene reached up to 10% here and was of the Benin haplotype (Nagel, 1984).

1.3.7.4 The Middle East and Arab countries

In these regions, SCD shows severe and benign forms that may be due to two β-globin gene haplotypes. The majority of patients in the Arab countries have the Arab-Indian haplotype (El-Hazmi *et al.,* 2011).

In his interesting literature review, El-Hazmi classifies the Middle Eastern Arab countries into three regions: the Gulf countries and Yemen; Palestine, Jordan, Syria, Lebanon and Iraq; and Egypt, Libya, Tunisia, Algeria, Morocco and Sudan.

Common disease patterns and frequency of occurrence of the HbSS gene in these countries have been studied extensively. Many reports have been published regarding different areas of each country (El-Hazmi *et al*., 2011).

SCA genes occur in two areas in the eastern and south-western areas of KSA. In the western provinces, the gene is linked to the Benin haplotype, while the Arab-Indian haplotype is found in the Eastern Province (Daar *et al.*, 2000; Serjeant and Serjeant, 2001). The severity of the disease is influenced by other genetic factors such as α-globin gene deletion and the HbF content. The α-thalassaemia disease occurs in 50% of the population in both the eastern and western areas of KSA.

A high level of HbF is characteristic of the Arab-India haplotype and could explain the milder clinical course that is observed in the Eastern Province compared with the south-western part of the KSA (Serjeant and Serjeant, 2001).

According to Diwani (1944), the first documented case of HbSS and thalassaemia in the Middle East was in Egypt. Lehmann (1954) reported the presence of HbSS in the Eastern Province of KSA; in Sudan, however, it was reported earlier.

1.3.7.5 Sudan

The presence of the HbS gene in Sudan was first reported by Archibald (1926) and later by Abbott (1950). There are some variations in the frequency at which this gene occurs within different tribes. Prevalence of SCT has been noted among 24% of new-borns and 29% of those over the age of five years. SCA patients present with severe complications and frequently fatal episodes in early childhood (Mohamed, 1992). Some of these children are diagnosed late and have already experienced severe complications.

In Sudan, SCD is considered one of the major causes of anaemia. It is a large health problem that places a burden on society and individuals. In Western Sudan, Nigeria and the western part of the KSA, the gene is common and SCT prevalence is high.

Two studies that investigated the prevalence of SCT among relatives of people with SCD and affected tribes were performed in Western Sudan and a third in the eastern region. All showed similar results. The study by Abdel Rahim and Attalah (1991) in the west of Sudan showed that SCA was predominant (68.4%) among Afro-Asiatic-speaking groups, which included nomadic groups of Arab and non-Arab descent that had migrated to Sudan at various points throughout history. Their research was similar to a study performed by Omer *et al*. (1972) of abnormal haemoglobin in the indigenous and immigrant tribes of Sudan. This study showed the highest number of SCT incidents among the immigrant tribes. As the majority of the study population (98.24%) was of a single ethnicity, there was a high prevalence of within-group marriage, which created a high risk that the sickle cell gene would be augmented.

A recent study was conducted in west Kordofan Province by the author and his colleagues,

*The epidemiology of sickle cell disease and determinants of **k**nowledge, **a**ttitudes, and **p**ractices (**KAP**) towards the disease in Western Kordofan State, Sudan* (2014). It was a community-based, descriptive cross-sectional study that was conducted in three towns in Western Kordofan State. A total of 372 households and about 1,112 individuals were included. Socio-demographic, socioeconomic and KAP data were collected through the use of investigator-administered questionnaires. Consanguinity was found to be the main cause of disease spread. The Messiria tribe constituted 50.9% of the study population, and consanguineous marriages were reported in up to 67.5% of the households (Daak *et al.*, 2016).

The highest proportion of homozygous SCD was 2.8% among children under five years of age. Carriers of the HbS allele constituted 24.9% of the study cohort. The average HbS frequency across all age groups was 14.5%. The most frequent β-globin gene cluster haplotype was Cameroon (30.8%), followed by the Benin (21.8%), the Senegal (12.8%), and the Bantu (2.2%) haplotypes. Considering all causes of child mortality, the percentage of childhood deaths due to SCD was 17.0% (Daak *et al.,* 2016).

Of the households surveyed, 46.9% were classified as having poor knowledge, 26.1% as having satisfactory knowledge and 26.9% as having good knowledge regarding SCD. The parents' educational levels were significant predictors of households being classified as having good knowledge about SCD ($p<0.05$). When asked about their attitude towards SCD, 48.0% were considered to have a satisfactory attitude, 30.7% had a poor attitude and only 21.3% expressed a good attitude. Poor knowledge of SCD and low socioeconomic status were the strongest predictors of poor attitude and practices towards SCD ($p<0.01$) (Daak *et al.*, 2016).

In the North Kordofan State city of El-Obeid, the prevalence of SCT among the relatives of SCA patients in the Bedaria tribe was reported to be 23.1%. This was followed by the Fulani and Selehab tribes, which had equal frequencies of 15.4% each (Mansour, 2011).

These tribes must be included when massive health education programmes or focus-group discussions are planned. A similar study conducted in eastern Sudan revealed an SCT prevalence of 66.7% among the population in Algedarif state (Lamiyaa, 2014). Among the subjects studied, HbAS was found in approximately 35% of the Hausa tribe and 24% of the Massaleet tribe, whereas HbSS was reported to be at a prevalence of 6% and 5% in the Hausa and Massaleet tribes, respectively (Sabahelzain and Hamamy, 2014).

In central Sudan, the prevalence of SCD is increasing in the Sudanese capital, Khartoum. This may be due to the movement of tribes from Western Sudan because of war and tribal conflicts that occurred between 1970 and 1980 (Sabahelzain and Hamamy, 2014). Most of the patients settle in Khartoum in a search for health services and regular follow-up visits at a haematology clinic that has been established there.

This clinic for sickle cell patients at the centre of Khartoum City provides all the necessary facilities and quality counselling. The late Dr Bakhita involved in much research regarding SCD and worked hard to establish this clinic. There has been much research on SCD there, and the facility now serves as a treatment and follow-up clinic for SCD patients.

1.3.7 Factors that influence the frequency of occurrence of SCD

Factors that influence the occurrence of the disease include consanguinity, migration and intermarriage within large tribes in African and Arab countries. Consanguinity is spreading in Sudan among the most-affected tribes of Messiria, Hausa, Bedaria and Massaleet. Environmental factors also contribute to the frequency of occurrence of the HbS gene, the most well-known example being malaria (Livingstone, 1957).

SCT confers a natural resistance against malaria, and this is a major advantage for survival in adverse conditions. It has been observed that HbS offers some protection against falciparum malaria. Sickling can occur in infected cells at normal oxygen tension. This relationship between malaria and the formation of sickle cells should be studied further to gain an understanding of a mechanism of protection against malaria (Gong *et al*., 2013).

The prevalence of asymptomatic malaria (caused by the parasite, *Plasmodium falciparum*) in SCA patients ranges from 27.3% when detected by microscopy to 47.4% when detected by polymerase chain reaction (Abiodun *et al.,* 2016).

1.3.8 Clinical manifestations of SCD

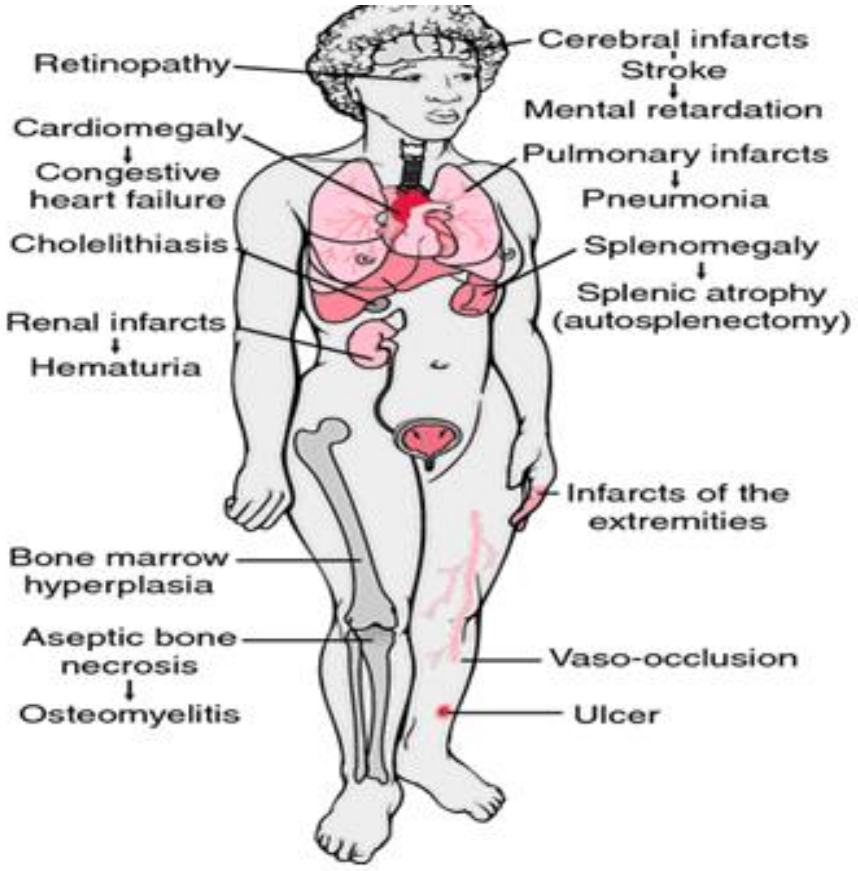


Figure 1-6 Diagram showing systemic complications of SCD.

(https://www.bio.miami.edu/dana/250/250SS18_8.html)

SCA can occur in a severe form with very low levels of haemoglobin. Some people with haemoglobin levels of 6-8g/dl may have mild symptoms without crises.

In symptomatic people, the disease can manifest in the form of painful swelling of the fingers, which is common in infants after six months of age; pain throughout the body, accompanied by pallor and weakness; haemiplegia; jaundice; sudden blindness or loss of vision; and hip pain and limping due to ischaemic necrosis of the femoral head. The fundamental pathological process in SCA is blood vessel occlusion, which is a form of vaso-occlusive crisis. Blood cells block the vessel lumen and interrupt blood flow to various parts of the body. This process

results in pain and damage to the brain, bones and other vital organs (Okpala, 1998; Serjeant *et al.,* 1994).

Those with silent cerebral infarction may show mild neurological deficits (Pegelow, 2001). These patients run very high risks of experiencing recurring strokes, even if they are not suffering from other illnesses (Wong and Powars, 2007). Children with SCA may suffer strokes or develop other disabilities, such as paralysis, aphasia or impaired cognitive function (Armstrong *et al*., 1996; Croft *et al*., 1993). The effects of the disease may be direct, such as an infarct, or indirect. Some children may develop blindness or fan-shaped haemorrhages that are detectable during routine eye examinations. In SCA, acute abdominal pain caused by ischaemia and obstruction of the mesenteric vessels with fever is an emergency that must be distinguished from acute appendicitis.

1.3.9.1 Chronic anaemia

RBCs in SCA behave normally in oxygenated conditions. But when oxygen levels are low, they tend to develop a sickle shape because, as their solubility falls, the cells aggregate and form liquid crystals. When oxygen becomes abundant, the shape of these cells returns to normal. Repeated sickling and polymerisation result in membrane rigidity and damage to the affected organ or the involved blood vessel. The sickled and damaged RBCs are then removed by the reticuloendothelial system, and this process usually leads to chronic anaemia. Haemoglobin levels range between 6.5 g/dl and 9.0g/dl in SCA patients and their bone marrow shows signs of erythroid hyperplasia (Rajab and Skerman, 2004).

1.3.9.2 Painful sickle cell crises

At birth, the HbF level is high, enabling the infant to live and grow without issues. HbF ($2\alpha\ 2\gamma$) in early infancy contains no β-globin. In SCA children, after six months in a homozygous state,

the β-globin genes become abnormal. SCA appears with symptoms of anaemia and painful crises when HbF levels are low.

Children, particularly when they are very young and sometimes only a few months old, often experience dactylitis of the hands and feet or 'hand-foot syndrome'. This symptom is painful and may result in premature closure of the affected epiphysis, which leads to the development of shortened, deformed bones (Serjeant *et al*., 1994). This process is part of a vaso-occlusive phenomenon (Serjeant *et al*., 1994). When swelling persists after the pain subsides, it should be thoroughly investigated to rule out osteomyelitis.

Dactylitis can be a cause of hospital admission. Painful limbs and acute abdominal pain may be the presenting symptoms. Priapism may also be present in male children. Severe headaches and vomiting with some visual disturbances are common. These patients require the application of magnetic resonance imaging (MRI) to diagnose silent infarcts. Abdominal pain may be localised to the upper right quadrant due to hepatic sequestration or a gallstone, or it may appear in the upper left quadrant due to splenic sequestration. Abdominal pain may be referred pain in the case of hip necrosis.

1.3.9.3 Infections

Patients with SCD have an abnormal immune system. The spleen may regress and disappear as a result of either frequent sequestrations or auto-splenectomy. Loss of splenic function predisposes the patient to increased susceptibility to infection by encapsulated organisms such as streptococcus pneumonia or salmonella. Such infection leads to sepsis or osteomyelitis. Before the advent of prophylactic penicillin use, children with SCD were prone to develop the invasive pneumococcal disease in the form of septicaemia or meningitis (Halasa, 2007).

Immune system abnormalities include impaired leucocyte function and problems caused by their failure to adhere to the endothelium (Okpala, 2004). There are also abnormalities of

complement, immunoglobulin and cell-mediated immunity (Serjeant and Serjeant, 2001). Complement abnormalities can affect both the classical and the alternative immunity pathways, due to the high turnover of factor B; defective opsonisation is also common in SCD (Wilson *et al*., 1979; Winkelstein and Drachman, 1968). Other bacterial infections may occur, such as *Staphylococcus aureus*. Treatment of acute crises includes the provision of oxygen, adequate fluids and broad-spectrum antibiotics with blood culture to support the diagnosis, and complete and differential blood counts.

A chest X-ray and measurement of C-reactive protein levels may be required for a complete diagnosis. Close observation may also be required to avoid deterioration or occurrence of shock or acute chest syndrome.

1.3.9.4 Acute chest syndrome

This condition is considered to be a life-threatening complication of SCD. It is the most common reason for admission to intensive-care units (ICU), for all age groups and usually presents with cough, fever, breathing difficulties and chest pain. The patient may have progressive anaemia and dyspnoea. They are hypoxic and appear ill and pale, with audible crepitations and wheezing. A chest X-ray shows infiltrates. This syndrome is thought to be due to red cell sickling in the lungs combined with recurring infection. However, the underlying causes are not yet clear.

Treatment consists of the provision of fluid for hydration and antibiotics for infection, the performance of blood culture and possibly blood transfusion if the haemoglobin level has dropped below the baseline. The patient may also need ventilation. Early detection and correct management may reduce the severity and prevent the patient's death. Clear improvement has been noted following blood exchange or transfusion and the introduction of heparin and antimicrobial agents (Serjeant and Serjeant, 2001).

The use of an incentive spirometer is very useful as a prophylactic during and after discharge from the intensive-care unit. These patients may have reactive airway disease or a history of asthma, so most of them benefit from the use of steroid inhalers (if started early) or bronchodilator inhalers. In severe cases, salbutamol and budesonide nebulisation may be required. Asthmatic patients with severe acute chest syndrome require early intubation and ventilation.

1.3.9.5 Endocrine and metabolic changes

Iron overload or vessel ischaemia may lead to endocrine complications. It is currently thought that iron overload is the main underlying cause of endocrine dysfunction in patients with SCD. Increased numbers of transfusions have been associated with the risk of endocrine organ failure.

Delayed pubertal development, gonadal failure, diabetes, carbohydrate intolerance and primary hypothyroidism have been documented. Diagnosis may be through the use of MRI rather than ferritin levels (Smiley, 2008). Generally, treatment consists of improved nutrition and the replacement of particular hormones. It is not clear whether patients with SCD are prone to endocrine pathology in the absence of iron overload, due to crises in the glands.

1.3.9.6 Other complications

Occasionally, splenomegaly or splenic sequestration, which is associated with profound anaemia and is due to the pooling of blood in the spleen, can occur in adults when splenic auto-infarction did not take place during childhood. Gallstone formation is common in SCA, secondary to increased red cell haemolysis, and can lead to the development of acute cholecystitis. Leg ulcers or learning difficulties may also occur. Haemolytic jaundice associated with SCD is sometimes fatal when native treatment is administered and the provision of professional medical assistance is delayed because it can cause a massive haemorrhage.

1.3.9.6.1 Renal Complications

SCD causes nephropathy in the form of acute kidney damage. Impaired urinary concentration, abnormal distal nephron function, haematuria and urinary tract infection are increased in SCT patients. These patients are also found to have an increased incidence of renal medullary carcinoma (Nath and Hebbel, 2015).

Individuals with SCT usually suffer renal dysfunction and develop the end-stage renal disease at a greater rate than those with normal HbAA blood in the black American population (Vimal *et al.,* 2010).

Patients with homozygous SCD have more severe renal complications than those with heterozygous conditions. Renal complications are associated with diminished life expectancy in SCD patients and lead to a 16-18% rate of mortality. This problem affects the healthcare system in terms of cost and leads to serious health issues for the individuals concerned (Nath and Hebbel, 2015). Death due to renal complications in adolescents affects the economic status of any country because it reduces the number of working adults. There is an interesting association in SCD cases among elevated pulse pressure, proteinuria haemolysis and chronic kidney disease (Novelli *et al*., 2014). This correlation may help in the early detection of cases to promote survival to adulthood.

1.3.9.6.2 Neurological complications

Clinicians should suspect cerebrovascular disease in sickle cell patients who have pulmonary hypertension. Some studies suggest that many physiological, epidemiological and clinical features of the arteriopathy of pulmonary hypertension overlap with those of stroke in SCD. These features are explained by abnormal intima, smooth muscle hypertrophy and thrombosis (Novelli *et al*., 2014). When these features occur with silent infarct, some children may lose their vision, become aphasic or develop a learning disability.

1.3.9 Growth and nutritional status in SCD

Studies performed on cases around the world have shown that SCD affects childhood growth and development. Although few studies have been conducted in Africa, the studies that have been performed have found that SCD affects all areas of endocrine, metabolic and micro-nutritional elements during growth and development (Al-saqladi, 2008). People with SCD are known to be shorter in height than normal individuals; their nutritional state is compromised by the disease and drug treatments.

Diagnosis of SCD:

To diagnose a case of SCD, all an individual's life details must be considered, including their tribe and family history. They must undergo a full, detailed physical examination with attention to organ involvement and potential referred pain. This examination must include the collection of a blood sample to test for sickling through the use of a solubility test and confirmation through the performance of haemoglobin electrophoresis with the use of High-performance liquid chromatography (HPLC), to determine whether the migration indicates HbAS, HbSS or HbAA. This test serves to quantify the amount of sickle haemoglobin and other types: HbAS (SCT) is diagnosed at haemoglobin S levels of 45% or less, and HbSS (SCA) at 65% or above. Family screening is also required for a newly diagnosed case.

Figure 1-7 Sickle Cell solubility test (Photo reprinted courtesy of Prof. S. Alkindy, Department of Haematology, Sultan Qaboos University Hospital, Oman).

SCD has been associated with other abnormal haemoglobins. For example, HbS-Oman has two mutations in the β-chains; in addition to the classic β-mutation (β6 glutamine-valine), it carries a second mutation in the same chain (β121 glutamine-lysine), which is identical to that found in HbO-Arab (Nagel *et al*., 1998). Family studies have revealed that consanguine marriage is involved in more than 56% of cases in Oman (Rajab *et al*., 2000); in our present study, this rate was 68% among SCT (HbAS) cases in Sudan.

Diagnosis can also be carried out post-conception or antenatal through foetal blood sampling and genetic studies (Edwards, 1993). This may help parents and clinicians to develop future plans that involve close follow-up of the child. A common practice is to repeat the test for confirmation at the age of six months, once the HbF level has dropped. A neonatal screening program is not yet started in Sudan for sickle cell disease.

1.3.10 Treatment options

Treatment of SCD in developing countries such as Sudan faces many problems. There is poor health education, which hampers the introduction of any new programme. Only 30% of the population has a good level of knowledge of SCD (Daak *et al*., 2016). In the west of Sudan, many people have misconceptions about the disease and follow whatever the tribe leaders say or practices they adopt. Sometimes, to treat haemolytic jaundice, they tattoo and burn the skin, because they believe this practice will remove the yellow discolouration of the skin and eyes. They also place small lacerations around the umbilicus in an attempt to relieve abdominal pain. They might apply other measures before asking for medical advice.

Figure 1.8 below, shows a picture of a 14-month-old child with swelling in his fingers. His mother applied some sand (seen as white patches in the picture) to the dorsum of his hands to cure the swelling and pain. The sand had been collected from the grave of a religious person, as his mother strongly believed that the application of such sand would cure the illness and stop the pain. We took him to the hospital to test for SCD and to receive conventional treatment. Blood samples from his parents were sent for testing; both parents were found to be carriers (HbAS). He was diagnosed with SCA (HbSS).

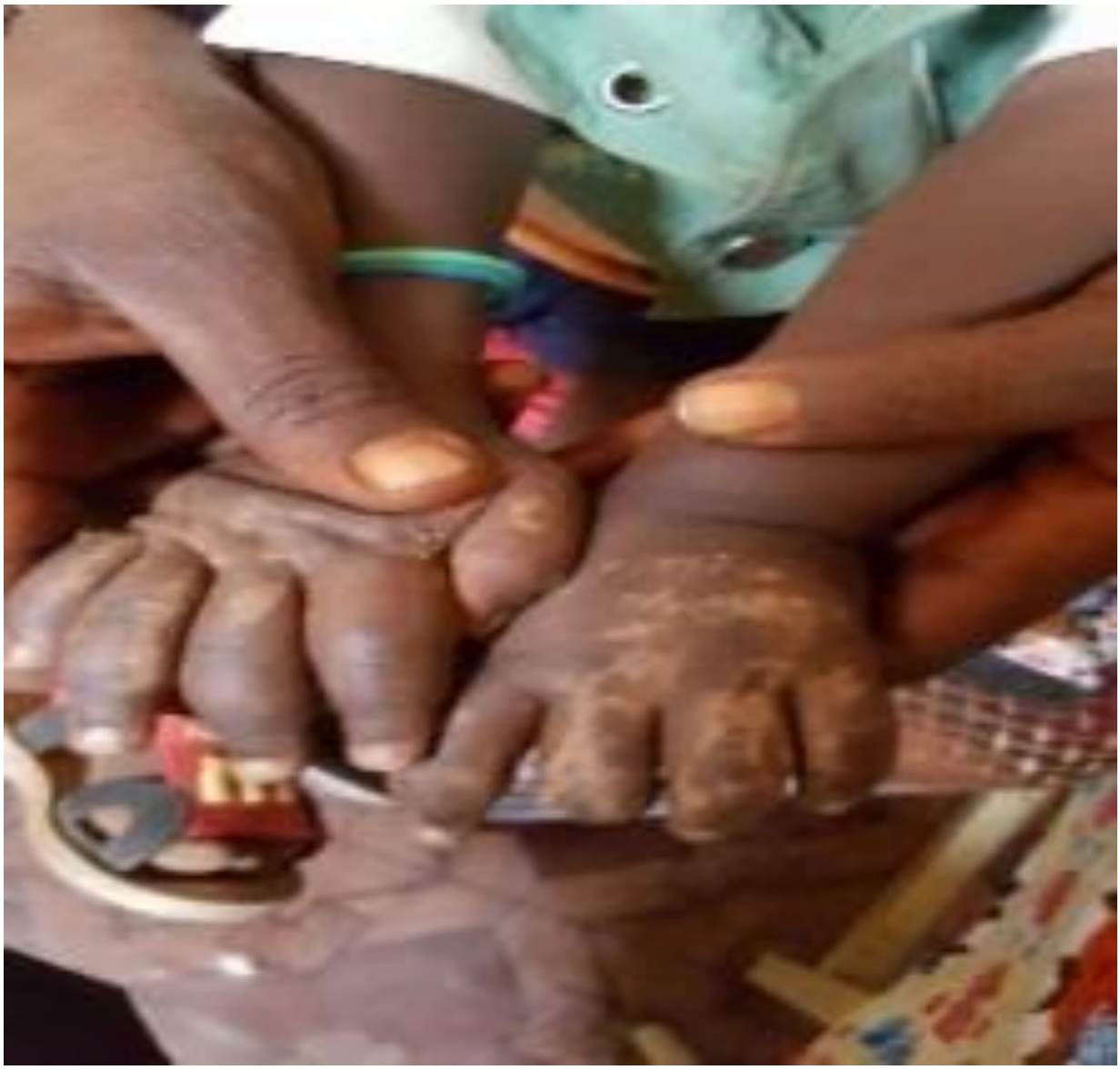


Figure 1-8 Local sand treatment for dactylitis (source: Tigani, 2017) during fieldwork

The main courses of disease management after an accurate diagnosis are pain killers and high-quality counselling. Folic acid is given as a micronutrient to aid in the production of RBCs in SCA and other types of anaemia. Sedatives should be avoided, but for those who are highly agitated or anxious, treatment may include anxiolytics such as benzodiazepines or even haloperidol. These treatments were provided with caution (Alli, 2014). Further management usually include intravenous fluid and ensuring adequate oral fluid intake. In severe cases, the

patient receive non-steroidal drugs followed by opiate therapy. Clinicians tend to proceed further and perform an early blood transfusion.

Recently, many patients have been found to improve through the administration of hydroxyurea, which increases levels of HbF and reduces the severity of the illness. Its use has been shown to reduce the frequency of occurrence of painful crises and hospital admissions and to cut the number of blood transfusions required. Its administration can also improve school performance and attendance (Ware *et al*., 2011).

The patient should be monitored carefully and closely through the performance of frequent blood counts. If bone marrow suppression is detected, the dose may be adjusted accordingly or stopped. Hydroxyurea is contraindicated during pregnancy and dosage should be stopped three months before conception.

L-glutamine is an amino acid that can be used to reduce the severity of acute crises in both children and adults. It also reduces hospital admission rates and the number of blood transfusions that patients require. It does not increase haemoglobin levels but the severity of symptoms can be markedly reduced. Administration of L-glutamine was observed to increase levels of other amino acids such as arginine (Quinn, 2018). Arginine is a precursor for nitrous oxide, which has a powerful effect on vascular tone. Its presence reduces the occurrence of vaso-occlusive crises (Cooper, 1977; Harbige *et al*., 1990).

A bone marrow transplant can be helpful to reduce the occurrence of severe recurrent crisis attacks and subsequent admissions, but the donor should be chosen carefully. Haematopoietic stem cell transplantation is curative for SCD (Leonard *et al*., 2020).

Anticoagulants and antiplatelet and thrombolytic agents are other treatment options (Roach, 2008). The efficacy and safety of these drugs have not been investigated in controlled trials.

Most high-risk patients in developing countries have no access to these treatment options and there is a major risk of infection. There is an urgent requirement for effective management to prevent overt strokes in these patients (Sarnaik *et al*., 1979).

The eyes should be examined regularly to detect any complications that can be treated by laser therapy. Pneumococcal vaccines for SCD are recommended and are effective in the prevention of infection (Davies *et al.*, 2004).

Omega-3 fatty acids have recently been administered to treat SCD patients because they improve lipid levels, and lipids are involved in red cell membranes and improvement of adhesion activities (Daak *et al*., 2011; Ghebremeskel *et al*., 1997; Ren *et al*., 2005a, 2005b). The goal is to prevent blood flow changes by reducing the risk that blood flow will decrease and preventing adhesion of sickled RBCs and plugs to small blood vessel walls. In so doing, the occurrence of both ischaemia and painful crises is reduced and hospital admission is avoided. Omega-3 fatty acids also act as antioxidants, preventing the accumulation of reactive oxygen species (ROS) and increasing levels of nitrous oxide so reducing the occurrence of vaso-occlusive crises.

Some patients may show renal involvement and develop renal failure, which results in a need for dialysis and renal transplant. Patients who suffer from hip necrosis may benefit from hip replacement therapy, which has advanced and is becoming a routine operation. A patient who suffers a stroke should be given early physiotherapy treatment. Those with gallstones can undergo operations to remove the gallbladder.

High-risk patients in developed countries, particularly children, are treated with periodic blood transfusions. The blood is usually well-screened and well-prepared and the patient is closely observed during transfusion. This therapy reduces the occurrence of initial and recurrent strokes

by over 80% (Gebreyohanns and Adams, 2004; Pegelow *et al.*, 1995). Unfortunately, blood transfusions are associated with high rates of transmission of infective agents, alloimmunisation, transfusion reactions, iron overload and other complications (Riddington and Wang, 2002).

During this survey, we found a paediatrician in western Kordofan who offered blood transfusions as an option to treat acute, severe crises, since other treatment methods were not available. He does not have a blood bank in his area to support him.

The protocol that was applied by the paediatric department at Al Nahda Hospital, where I worked (Oman, 2008-2015) was to admit patients with severe vaso-occlusive crises who did not respond to simple analgesics. These patients were usually given morphine or tramadol and intravenous fluids. Some of them experienced severe pain and required blood transfusions. Most of the children responded well and were able to go home after a few days.

SCA accompanied by a fever is also a cause for admission and the start of a course of antibiotics that continues until the culture results are free from bacterial growth, or the patient is afebrile for 48 hours. Some patients arrive with acute splenic sequestration crises, severe pallor and a drop in haemoglobin level to below 5g/dl from a baseline of 6-9g/dl. These patients require blood transfusions urgently. Upon discharge, these patients are given folic acid supplements and oral penicillin as well as advice to have their spleens removed and to obtain the pneumococcal vaccine.

Psychological support and sex counselling are also provided by some clinics. Close follow-up and Doppler ultrasound studies of the head and neck are helpful in the detection of early cerebral infarct or the risk of it.

1.3.11 Red cell adhesion and vaso-occlusion

Sickle cells adhere abnormally to the endothelium (Hebbel, 2008; Hoover *et al.*, 1979). How tightly RBCs adhere to the blood vessel walls correlates inversely with the size of the venular capillaries, and the prime site of adhesion is close to the post-capillary venules (Kaul *et al*., 1989; Kaul and Fabry, 2004; Zennadi *et al*., 2007). Studies have revealed that RBC adhesion acts as a trigger that slows down the blood flow to fulfil the delay time required for sickling (Hebel *et al*., 1980; Kaul and Fabry, 2004).

This finding may help to explain the established positive correlation between the adhesiveness of sickling cells to endothelium and the clinical severity of SCD (Hebbel *et al*., 2005). Several factors play a role in this adhesiveness. The adhesion molecules that are commonly expressed in sickle cells include very late antigen-4 and α4/β1 integrins (Brittan and Parise, 2008; Joneckis *et al*., 1993).

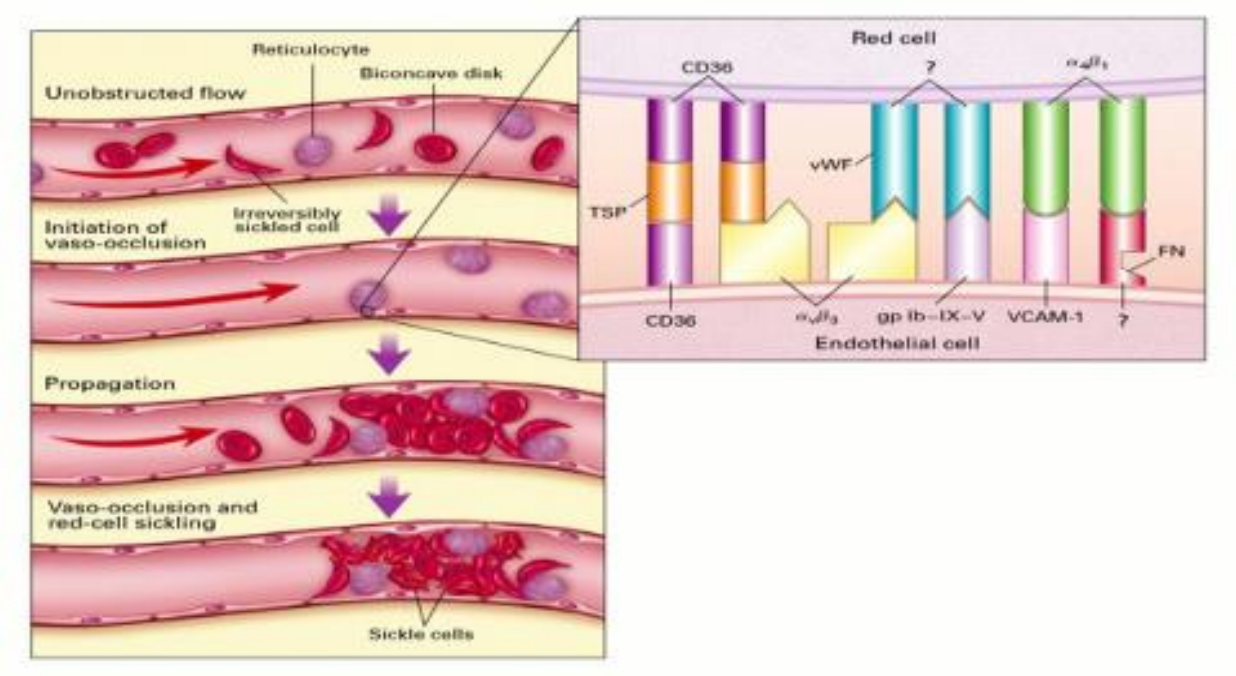


Figure 1-9 Diagram showing how slow-speed sickling and endothelial adhesion contribute to vessel blockage. (Adopted from Frenette and Atweh 2007)

White blood cells are thought to play a critical role in the pathophysiology of vaso-occlusion in SCD, because they show an increased propensity to adhere to the vascular endothelium and RBCs (Canalli *et al*., 2008; Frenette, 2002, 2004). Platelets play a role in vaso-occlusion and hypercoagulability in SCD. Other factors regarding these topics have been studied and documented, including the prothrombotic state, anticoagulant proteins, increased tissue factor procoagulant activity and plasma P-selectin (Chiang and Frenette, 2005).

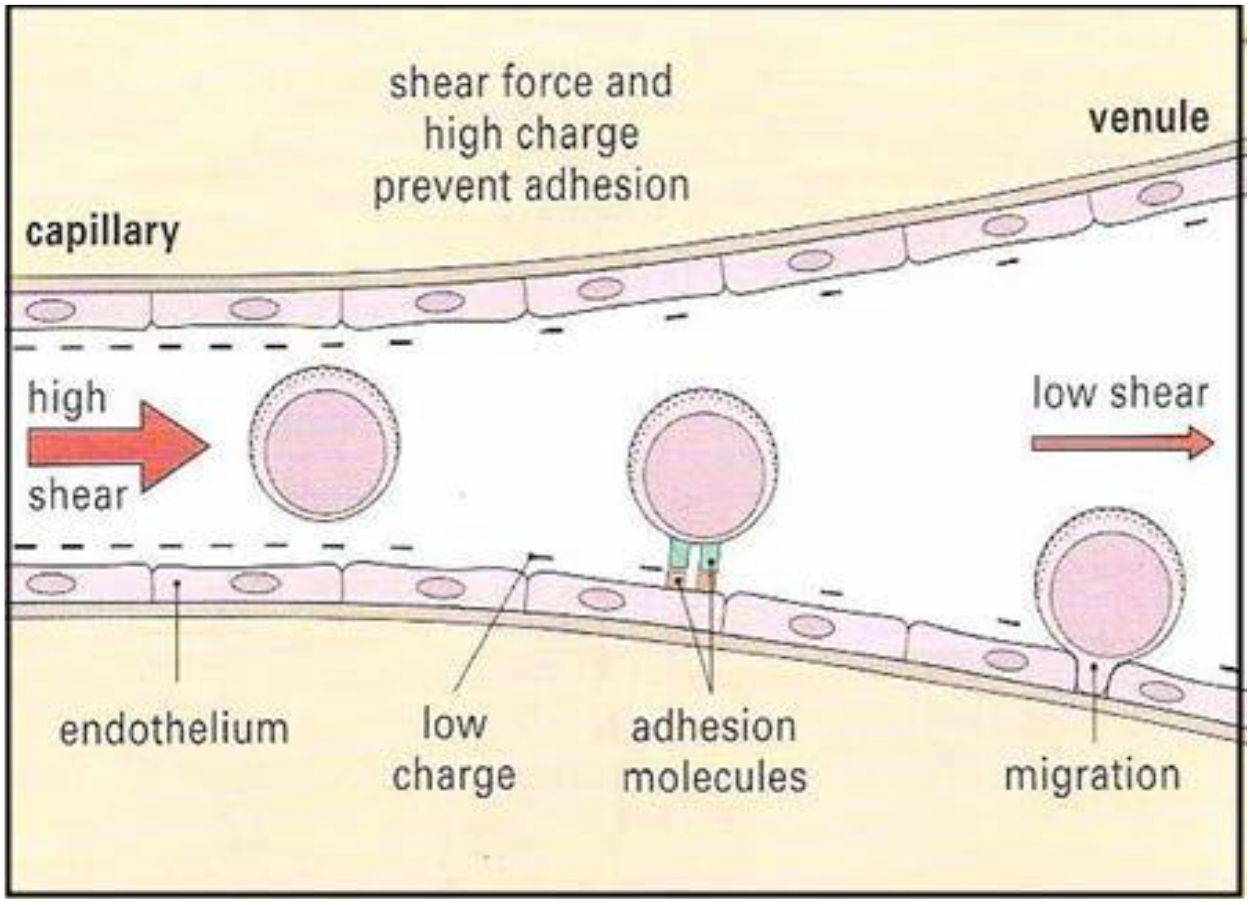


Figure 1-10 Leukocytes migrate in blood vessels from high to low shear force as adhesion molecules are expressed (adapted from Roitt et al., 2002).

1.3.12 Cell membrane and SCD

It is recognised that cell membrane defects in the sickle cell have a significant influence on the pathophysiology and clinical severity of the disease (Hebbel, 1991). The major membrane abnormalities that are observed in SCD are a defect that compromises membrane transport (Gibson and Ellory, 2002), a dysfunctional lipid bilayer (Barber *et al*., 2009; Kuypers, 2007) and abnormalities in the fatty acids of the blood cell membrane (Ren *et al*., 2006). Dysfunctional lipid bilayers in the sickle cell membrane are characterised by the externalisation of serine phosphoglycerides (Balasubramanian *et al*., 2007; Kuypers *et al*., 1996; MiddelKoop *et al*., 1988). Lipids are involved in the highly specialised process of signal transduction. Experimental and human studies demonstrate that abnormal composition of and deviations from normality among lipids lead to cell dysfunction.

Vaso-occlusive crisis in SCD was thought previously to be the result of a mechanical blockage of small blood vessels by rigidly distorted (sickled) RBCs. Relationships were observed between the formation of some irreversible sickle cells and vaso-occlusive events (Zipursky *et al*., 1993). Blood cells of sickle cell patients are more likely to adhere to vascular endothelium than the blood cells of people without SCD, and there is a correlation between the amount of these adhesive interactions and the occurrence of vaso-occlusive crises (Chiu *et al*., 1981; Haynes and Obiako, 2002; Hebbel *et al*., 1980; Ohnishi *et al*., 2000; Schwartz *et al*., 1983). The current understanding is that the excessive tendency of RBCs (sickled and non-sickled) to adhere to vascular endothelium and the activation of platelets and leucocytes are the primary causative factors of vaso-occlusion (Frenette *et al*., 2002, 2007; Kaul *et al*., 1996; Okpala, 2006; Rosse *et al*., 2000).

Adhesion, aggregation and elasticity of blood cells and inflammatory response are strongly modulated by the actions of cell membrane fatty acids (Cooper, 1977; Harbige *et al*., 1990; Meydani *et al*., 1991; Mills *et al*., 1993; Hive *et al*., 1993; Nishiyama *et al*., 2000). Our group and others (Connor *et al*., 1997; Daak *et al*., 2011; de Jong *et al*., 2001; Ghebremeskel *et al*., 1997; Hebbel, 1984; Manodori *et al*., 2000; Muskiet and Muskiet, 1984; Ren *et al*., 2005a, 2005b; Wood *et al*., 1996) have reported findings regarding abnormal membrane lipids in steady-state patients with SCD. These abnormalities are characterised by enhanced translocation of phosphatidylserine from the inner to the outer leaflet of red cell-membrane lipid bilayers, increased levels of arachidonic acid (AA, C20:4n-6) and decreased levels of linoleic acid (LA, C18:2n-6), eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) in the red cells, platelets and mononuclear cells.

The above findings have led to the postulation that an imbalance of omega-3 and omega-6 (n-3 and n-6) fatty acids in the blood cell membrane may be the antecedent of loss of membrane asymmetry, blood cell dysfunction and vaso-occlusion in SCD.

Indeed, exploratory pilot studies (Golfetto *et al*., unpublished; Okpala *et al*., 2011; Tomer *et al*., 2001) showed that supplementation of sickle cell patients with n-3 fatty acids significantly reduced the number of vaso-occlusive crises that led to hospital admission.

In line with these exploratory studies, a placebo-controlled, double-blind trial that involved 140 Sudanese sickle cell patients demonstrated that n-3 fatty acid treatment ameliorated the lipid abnormality of blood cell membranes that were seen in these patients and reduced the number of vaso-occlusive crises that they underwent and which required hospitalisation (Daak *et al*., 2011; Ghebremeskel *et al*., 1997; Ren *et al*., 2005a, 2005b).

Other investigations have revealed that the degree of SCD abnormality that is caused by these perturbations of blood cell membrane n-3 and n-6 polyunsaturated fatty acids (PUFA) varies among patients from different countries. This suggests that the anomaly may be caused by a genetic component and modified by environmental factors, and severity may depend on the haplotype (Weiss *et al.,* 2012). The AA (omega-6) and DHA (omega-3) PUFAs are vital structural and functional components of neural, vascular and visual systems. AA and DHA are in high demand during pregnancy for the development of the foetal brain, blood vessels and other vital organs. It is estimated that the foetus accumulates about 70mg/d of omega-3 fatty acids, primarily DHA, and a comparable amount of omega-6 during the third trimester. Since the foetus has a limited ability to synthesise AA and DHA, it obtains them from the maternal circulation through placental selection. As this placental transfer increases the blood levels of AA and DHA in the foetus, it causes a concomitant decrease in the mother (see Figure 1.11) (Daak *et al*., 2011; Ghebremeskel *et al*., 1997; Ren *et al*., 2005a, 2005b). Even in seemingly 'well-nourished' healthy women, the blood levels of both AA and DHA decline as the pregnancy progresses. Sickle cell patients have low blood levels of EPA and DHA. Surprisingly, there is no published data on the effect of SCD on the transfer of essential fatty acids from the mother to the foetus. However, it is known that the transfer of AA and DHA from the mother to the foetus is impaired in mothers with chronic diseases such as diabetes (see Figure 1.12) or with conditions such as impaired fatty acid metabolism and placental infarction.

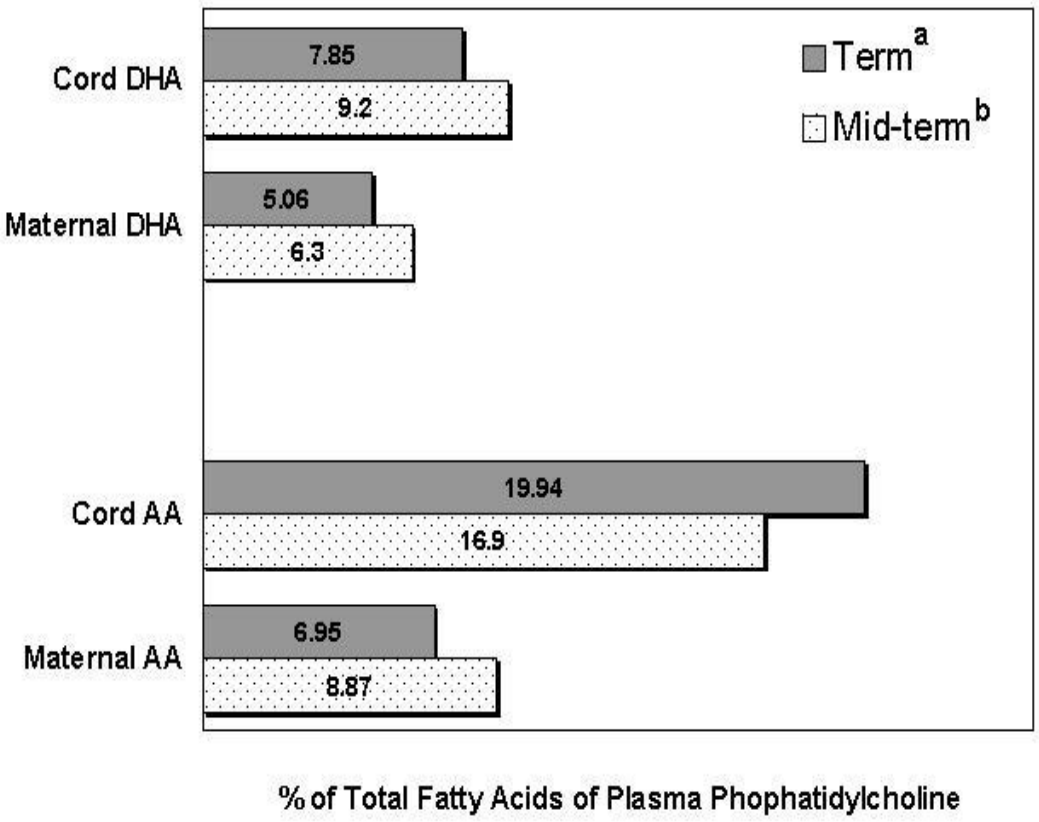
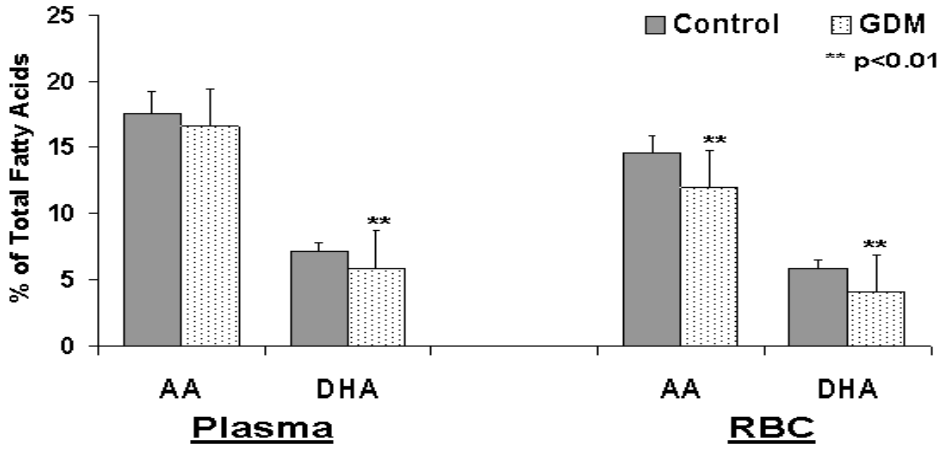


Figure 1-11 Levels of maternal and foetal (cord blood) arachidonic (AA) and docosahexaenoic (DHA) acids in plasma at mid-term and term.

(Handbook of Lipids in Human Function: Fatty Acids, Oxford: Academic Press and AOCS Press, 2015).

(a) Plasma and red cell (RBC) choline phosphoglycerides AA and DHA of neonates of non-diabetic (control, n=33) and gestational diabetic (GDM, n=40) women.



(b) Plasma choline phosphoglycerides AA and DHA of neonates of non-diabetic (control n=33), type 1 (n=26) and type 2 (n=15) diabetic women.

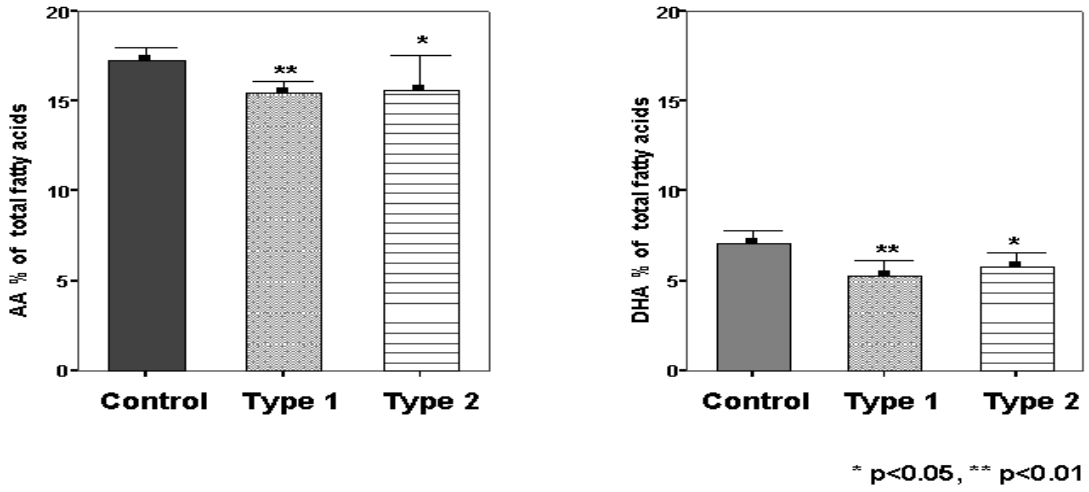


Figure 1-12 Neonates of diabetic mothers have reduced blood levels of arachidonic (AA) and docosahexaenoic (DHA) acids.

(Handbook of Lipids in Human Function: Fatty Acids, Oxford: Academic Press and AOCS Press, 2015).

1.3.13 Sickle Cell Disease and fatty acids

Studies conducted by our group and others over the last few years have demonstrated that patients with SCD have abnormal fatty acid compositions in plasma, erythrocytes and mononuclear cells. They are characterised by increased levels of omega-6 (AA) and significantly decreased levels of omega-3 (DHA and EPA) (see Figure 1.13). This abnormality, which is unrelated to dietary intake, explains the enhanced expression of adhesion molecules on white blood cells and the propensity of RBCs to aggregate and adhere to the vascular endothelium in SCD.

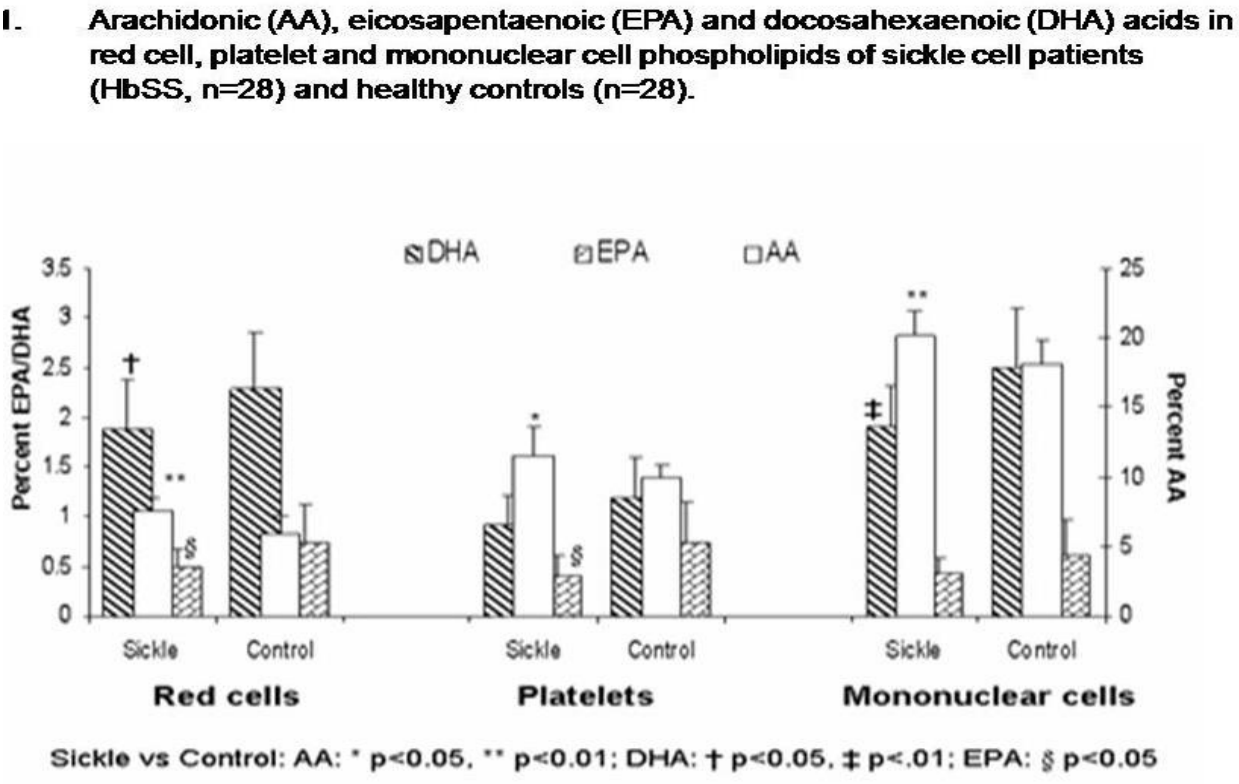


Figure 1-13 Diagram showing differences in levels of AA, EPA and DHA fatty acids in RBCs, platelets and mononuclear cells of patients with SCD compared with healthy controls

(Handbook of Lipids in Human Function: Fatty Acids, 2015).

1.3.14 Pregnancy and Sickle Cell Disease

People with sickle cell conditions who decide to have children require information regarding the expected complications during pregnancy and childbirth. For example, Ugboma and George (2015) have reported that pregnant women with SCT experience higher incidences of vaso-occlusive crises than SCT women who are not pregnant, and they face increased risks of preterm delivery, Caesarean section and postpartum haemorrhage than pregnant women without SCT. Pregnant women with SCT have an increased risk that complications may arise during labour that requires surgery or may result in infection (Hamdi *et al*., 2002).

Foetuses of pregnant women with SCT are at increased risk of hypoxia, according to Manzar (2000). When compared with normal HbAA pregnant women, they are reported to have more recurrent urinary tract infections and greater incidences of haematuria and foetal distress during labour (Tuck, 1983). Pregnant women with the trait are more susceptible to bacteriuria (Baill, 1990) and tend to have more postpartum complications within the first two weeks after the birth, such as severe infection and postpartum haemorrhage than women without SCT (Khandale and Kedar, 2015; Sonawane *et al*., 2005).

SCA with pregnancy is linked with some maternal complications such as vaso-occlusive crises, which occur in 30-50% of pregnant women with SCA, infection of any kind from mild to severe, which occurs in up to 28% of these women, anaemia in approximately 34%, pre-eclampsia in up to 9% and premature labour in up to 24%. Foetal or neonatal complications, including miscarriage, have been reported to occur in 24% of cases of pregnancy that involve SCA, low birth weight in 20% of these cases, foetal distress in 14%, and stillbirth in 0.9% (Alli *et al*., 2014).

A study conducted in Bahrain concluded that 60.6% of admitted pregnant women had homozygous SCD with raised levels of foetal haemoglobin (more than 5% HbF). Vaso-

occlusive crises were the main cause of admission of pregnant women with SCD in Bahrain, 28% of which were due to haemolytic crises (Rajab *et al*., 2006).

Clinical colleagues in Oman have reported an increased incidence of anaemia, miscarriage, and neonatal death among pregnant women with SCT when compared with pregnant women who have no sickle cell conditions (Hamdi *et al*., 2006).

In their 2006 study, Taylor and colleagues observed that there was an increased incidence of foetal loss, preterm delivery, and miscarriage among pregnant women with SCT when compared with normal pregnant women. They also found that all placentas of SCT women demonstrated sickling in the intervillous space and that sickling was found in the decidual vessels, especially in the draining of venous channels (Taylor, 2006).

A large meta-analysis study was conducted by Oteng-Ntim *et al*. (2014) that compared the birth outcomes for pregnant women with sickle cell conditions with those for women without sickle cell effects. The results indicated that SCA pregnant women and their babies were at high risk of mortality, stillbirth, low birth weight, and miscarriage, despite the efforts of obstetricians and neonatologists.

A study was conducted in India in which the admissions of pregnant women with SCD, SCA, or SCT between 2011 and 2015 were examined. It was observed that 1.2% of these women had SCA and 15.6% had SCT. Stillbirth deliveries represented 9.9% of sickle cell deliveries, compared with 4.2% of normal deliveries. More than 70% of deliveries to sickle cell mothers had a low birth weight as opposed to 44% of normal deliveries. It was also found that 50% of sickle cell deliveries required a blood transfusion and 45% were preterm, compared with 17% of normal deliveries that were preterm. Rates of severe anaemia, stillbirth, blood transfusion,

Caesarean section, and low birth weight were all significantly higher in SCA deliveries when compared with normal deliveries (Desai *et al*., 2017).

People with sickle cell conditions should be given information to make them aware of and help them to manage these risks.

Health care providers should take into account not only the clinical aspects of SCD but also the patients' psychosocial status through the provision of high-quality counselling on sexuality, reproduction, and genetics, to give this population the greatest chance of a good quality of life (Juliano *et al*., 2013). Young people can be offered health education, pneumococcal vaccination, and even advice on partner selection. People with SCD or SCT who decide to have children require information before they achieve pregnancy; for instance, patients on hydroxyurea should stop taking the drug at least three to six months before conception. Hydroxyurea is contraindicated during pregnancy. Patients can be advised regarding the potential termination of pregnancy. They can be informed that antenatal diagnosis is possible, whether through chorionic villous sampling between nine and 13 weeks, amniocentesis at 15 to 16 weeks, or foetal blood sampling at 18 to 20 weeks of gestation (Alli *et al*., 2014).

Advances in medicine have improved the situation for people with sickle cell conditions as they plan pregnancies. Advances in medicine mean that pregnant women with sickle cell conditions can be closely monitored and followed up until delivery. Many hospitals implement a multidisciplinary team approach and prepare well for such cases. A decrease in mortality has been noted after the application of these measures. However, in rural areas of Sudan, these improvements have not been widely introduced.

Care and follow-up of SCA and SCT pregnant women can be conducted smoothly to cover planned visits to the antenatal clinic. Delivery can be arranged and blood transfusions can be

given when indicated at any time during pregnancy, at delivery, or in the postnatal period. Surgical stress, labour effort, and bleeding are all causes of hypoxia, which may lead to intrapartum crises that require high doses of morphine but which place the infant at risk of respiratory distress after birth. A paediatrician should be called to attend the delivery and manage any drug effects on the infant (Alli *et al*., 2014).

After delivery, the mother should be advised to take adequate fluids and fresh air to ensure strong breathing, and to wear stockings to avoid crises and thromboembolic episodes. Blood can be taken from the umbilical cord for diagnosis. The infant can be placed in a follow-up program that involves repeat blood tests after six months. Blood transfusion issues for SCD or SCT patients during and after pregnancy must be taken seriously. Some may need exchange transfusion during acute crises to avoid fluid overload and to keep the percentage of HbSS below 30% (Alli *et al*., 2014).

Table 1-2 Indications for blood transfusion during pregnancy in SCD (Alli et al., 2014).

Acute	Chronic
Symptomatic anaemia Splenic/hepatic sequestration crises Acute chest syndrome Acute neurological syndrome Preoperative (major surgery) Acute multiorgan failure Sepsis	Primary or secondary stroke prevention Pulmonary hypertension Recurrent sequestration crises Chest syndrome Anaemia associated with chronic renal failure Congestive cardiac failure

Many of these advances have not been introduced in Sudan. For instance, so far, the nutritional effect of SCD and SCT on pregnant women has not been extensively studied in the country.

Most studies that have been conducted have been retrospective and focused on the pregnancy outcome and general health of the foetus or mother.

1.4 Rationale for this study

As noted, most studies have shown that SCD, SCT, and normal pregnant women experience different pregnancy outcomes. Pregnant women with SCA and SCT experience high incidences of premature delivery, stillbirth, anaemia, and blood transfusion. Essential fatty acid levels have not been reported in pregnant SCT patients. In an attempt to find the reasons for differences among the above groups, the present study measured levels of omega-3 and omega-6 fatty acids in women with SCT during pregnancy and in healthy controls in the first trimester and delivery.

Fatty acids are essential for brain and vision development, and there is a high requirement for omega-3 fatty acids during pregnancy. The foetus is dependent on the mother for the supply of these fatty acids, so if the mother is depleted, the baby also will be deficient. If pregnant women are deficient in these acids, it is safe for them to take supplements.

During pregnancy, it is expected that vitamins D, E, and β-carotene, and essential fatty acids, will be depleted. It has been reported that vitamin E supplementation increases levels of plasma and red cell tocopherol (Natta *et al*., 1980). Also, transfusion of packed RBCs may increase cell volume, haemoglobin concentration, foetal haemoglobin levels, and blood flow, and decrease the amount of irreversible lysis that occurs in sickle red cells (Muskiet *et al*., 1991). Similarly, dietary supplementation with the antioxidant nutrient zinc has been shown to increase levels of lymphocytes and granulocytes and to reduce the number of irreversible sickle RBCs and the rates of occurrence of vaso-occlusive crises and infections (Prasad *et al*., 1999).

1.4.1 Aims

This Ph.D. research programme aimed to investigate:

- The haematological and nutritional statuses of non-pregnant women of childbearing age who had SCA;
- The maternal and foetal outcomes in pregnancy that were complicated by the presence of SCT;
- Changes in levels of essential fatty acids during pregnancy in women with SCT.

1.4.2 Methods

1. Non-pregnant women of childbearing age with SCA (HbSS status n=39) and without, i.e. healthy controls (HbAA, n=36) were recruited from a sickle cell clinic in Khartoum, Sudan.

- Detailed demographic, clinical, and haematological data were collected from both groups.
- Blood samples taken from both groups at recruitment were analysed for haematological variables.
- A questionnaire developed for the study was used to assess food types that were regularly consumed in the households of the participants.

2. Pregnant women with SCT (n=34) and healthy pregnant controls (n=60) were recruited from El-Obeid Teaching Hospital (El-Obeid, North Kordofan, Western Sudan) during their first antenatal visits.

- Demographic, clinical, haematological, obstetric and birth outcome data were rigorously documented.
- Blood samples were collected from the women at recruitment and delivery, and from the babies at birth (cord blood), and these were analysed for haematological parameters.

- The blood specimens from the women were also assayed for essential fatty acid content.
- Information regarding the food types that were frequently consumed by the participants was obtained with the use of a questionnaire and these data were assessed

Chapter 2

2. Materials and Methods

2.1 Methods

This study was a hospital-based, case-controlled, cohort observational study (Wacholder *et al.*, 1992).

2.2 location of the study.

The area of study was El-Obeid city in Western Sudan. El-Obeid is about 600km southwest of the capital Khartoum. It is the capital of Northern Kordofan State. The area of the state is 71,546 square miles, populated with 2,920,890 people. El-Obeid city is an important commercial and stock market for Arabic gum, peanuts, and other agricultural products. The people of North Kordofan, like those of other states, suffer from many health problems which are exacerbated by tribal conflict, drought and floods that usually occur during July and August. There is malnutrition, poverty and a loss of productivity (United Nations Children's Fund (UNICEF), 1999; UN, 2000), all these affect the health of people in the region.

2.3 Subjects and recruitment

2.3.1 Group 1 (non-pregnant women)

2.3.1.1 Subjects

Women with steady-state (HbSS, n=39) and without (HbAA n=36) SCA were recruited during routine visits to the haematology clinic at the University of Khartoum Ibn Auf Teaching Hospital.

- **Inclusion criteria:** non-pregnant women aged 16-40 years who volunteered to participate in the study and had the mental competence to give informed consent.
- **Exclusion criteria:** any sickle cell crisis, acute illness or blood transfusion in the previous four months, presence of other chronic diseases, a physical disability which impaired

access to food or restricted eating, and/or pregnancy. Steady-state was defined as an absence of sickle cell crisis or acute illness in the four months before and up to two weeks after blood collection for the study.

2.3.1.2 Methods

Demographic data and medical history were collected from patients and hospital records with the use of a questionnaire developed for the study. Anthropometric measurements–(weight in kilograms and height in centimetres) was assessed through the use of a Seca Electronic Scale 890 (UNISCALE, Seca, Birmingham, UK) and a height measuring board (Shorr, Olney, Maryland, USA), respectively. Blood specimens, 5ml, were obtained from each participant.

2.3.1 Group 2 (pregnant women)

2.3.2.1 Subjects

The second group for this study comprised pregnant women with SCT (HbAS) (n=34) after the screening program, and a comparison, age-matched group of pregnant women without SCT who acted as healthy controls (HbAA) (n= 60).

- The inclusion criteria for this group were: age 18-40, mental competence to give informed consent, and expected to deliver in the city.
- The exclusion criteria were: SCA, thalassaemia, other chronic diseases, and a physical disability that restricted access to food or eating, and/or malnutrition.

2.3.2 Screening

A large group (n=367) of pregnant women was recruited from the obstetrics department of El-Obeid Hospital, Kordofan, Western Sudan. They were recruited during their first trimester and each was screened for SCT (HbAS). Of these women, 34 were found to have SCT and were enrolled in the study. The remaining 333 who did not have SCT were assessed, and 60, who fulfilled the inclusion and exclusion criteria and consented to participate in the study, were

enrolled. Fifty did not meet the inclusion criteria and 223 participated in the screening programme but were not willing to continue in the study for various reasons.

2.3.3 Methods

Detailed demographic, obstetric, medical history, dietary habit and birth outcome data were meticulously documented from these women in the baseline study. Blood samples (5ml) were collected for haematological analysis. The participants were followed until their expected date of delivery and contacted. At this point only 23 of the women with SCT could be traced and 40 of the healthy control group. From these remaining participants, blood samples (5ml) were taken and processed to examine their haematological status. Baseline blood samples (5ml) for fatty acid analysis were taken from 17 pregnant women with SCT and 20 pregnant healthy controls. Seventeen of the women with SCT and 20 of the healthy controls refused to give blood for fatty acid analysis. At delivery, blood samples (5ml) were taken for fatty acid analysis from 15 women with SCT and 15 healthy women. Of the rest, two women with SCT who had miscarriages and six others refused to give blood, while one of the healthy controls who had a miscarriage and 24 others in that group also refused. Anthropometric data (weight and height) were assessed through the use of .a Seca Electronic Scale 890 (UNISCALE, Seca, Birmingham, UK) and a height measuring board (Shorr, Olney, Maryland, USA), respectively.

2.4 Blood sampling and processing

Venous blood (5ml) was taken from each participant in both groups of the study in tubes that contained an anticoagulant, ethylenediaminetetra acetic acid (EDTA, K2 EDITA spray-dried, BD Becton Dickinson Company). A minimum amount (<1ml) was used for screening and then the remaining sample was processed for complete blood count and centrifuged to separate plasma and RBCs.

On the same day of blood sample collection, separation was performed by centrifugation at 3000 rpm (g) for 15 minutes at room temperature 27 ^{0} Celcius. The plasma (top layer) was carefully removed and transferred into a micro tube. The lower red cell layer was washed three times with physiological saline (0.85% NaCl) and centrifuged at 3000 rpm (g). The RBCs and plasma were frozen at below -40°C and transferred in dry ice to the London Metropolitan University research laboratory, where they were kept at < -80°C.

Blood collection was done in February 2017 and the plasma and RBCs were analysed for fatty acid content in February 2020 at the London Metropolitan University Science Centre. Each analyzer used was checked for quality assurance before the analysis according to the standard.

2.5 Determination of haemoglobin concentration and blood parameters

Haemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular levels of haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total white blood cell count (TWBC), and counts of neutrophils, lymphocytes and platelets (PLTs) were obtained through the use of an automated haematology analyser, the SysmexKX-21N (Sysmex Corporation, Kobe, Japan). HbS was quantified using the haemoglobin capillary electrophoresis machine (Minicap Sebia Flex Piercing, Lisses, France).

2.6 Principles of capillary electrophoresis

Capillary electrophoresis (CE) involves the performance of electrophoresis in buffer-filled narrow capillaries, 25-100μl in diameter. The separation relies on differences in the speed of migration (migration velocity) of ions or solutions, but the vitally important feature of CE is the bulk flow of liquid through the capillary, which is called electric osmotic flow (EOF). The inside surface of the capillary is coated in ionisable silanol groups, which readily dissociate to produce a negative charge on the capillary wall. The negative charge attracts the positively

charged ions from the buffer and an electrical double layer is created in which there are potential differences close to the capillary wall. When a voltage is applied across the capillary, cations in the diffuse layer are free to migrate towards the cathode, and these carry the bulk solution with them. The result is an EOF and separation of the differently charged haemoglobin fractions.

These fractions are detected directly at an absorbance wavelength of 415nm, which is optimised for haemoglobin, in the following order from cathode to anode: HbC, A2, E, S, D, F, A, Bart's, J, and H (Oyaert *et al.*, 2015).

The Minicap Sebia Flex Piercing instrument (Figure 2.1) could be programmed to perform all sequences automatically to show the final, clear-cut profile of the sample contents, with precise quantification and presumptive identification of the most common haemoglobins. The Minicap instrument enabled the high-resolution separation of the major haemoglobin variants (HbA, HbS, HbC, HbD, and HbE), and accurate quantification of the HbA2 and HbF. HbH and HbBart's were separated and easily quantified.

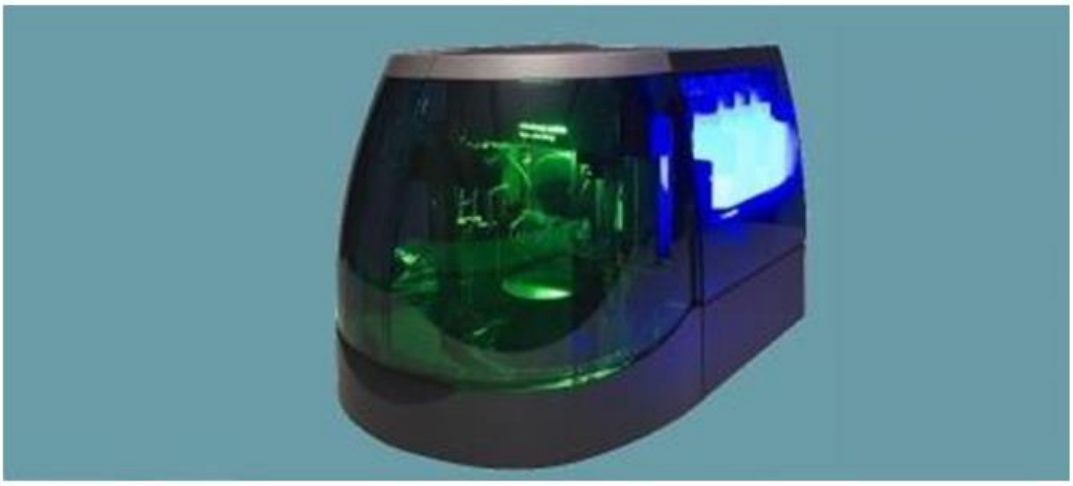


Figure 2-1 A photograph of a Minicap Sebia Flex Piercing electrophoresis machine.

2.6.1 Electrophoresis results and interpretation

The samples were put in a rack and the control was prepared according to the manufacturer's instructions. The machine was connected to a desktop, and when the run had ended, the results were analysed through clicks on each curve, which were then labelled according to the sample code. The machine recognised automatically the A_0, F, C, and A_2 peaks. Other peaks fell in a specific position (zones), for instance, HbS, HbD, HbE, HbH and HbJ, but they were all labelled as Hb**X**. A picture of an example screen is shown below.

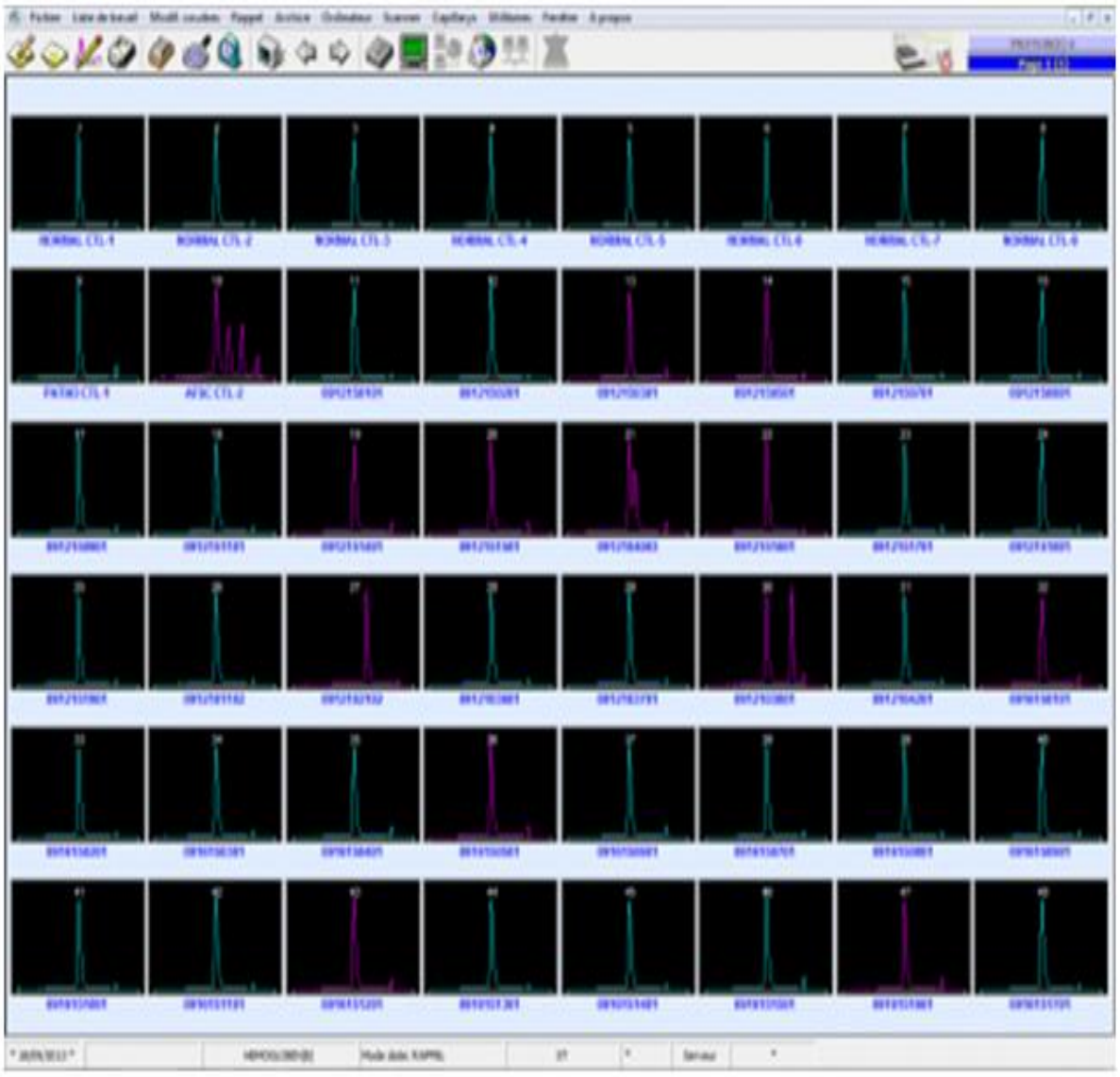


Figure 2-2 Screen shot of the desktop picture seen after the run was over.

The machine calculated the percentage of each type of haemoglobin that was present in the sample and the electrophoresis curve was shown as follows for both SCT (HbAS) and healthy control (HbAA) haemoglobins (Figures 2.3 and 2.4).

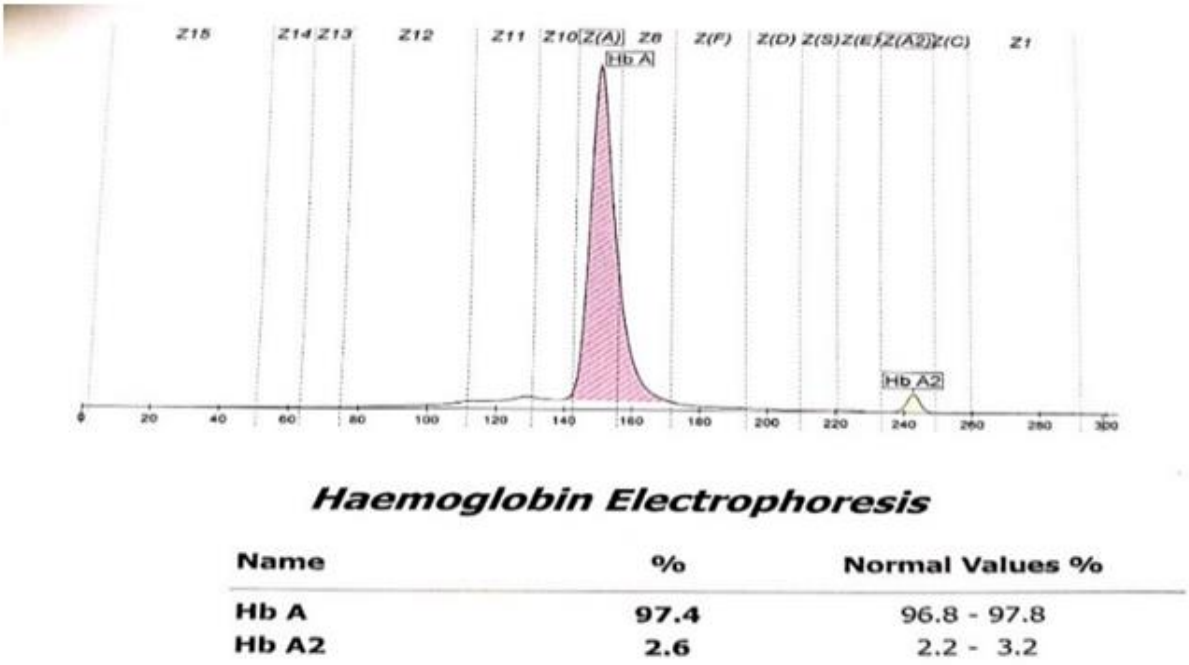
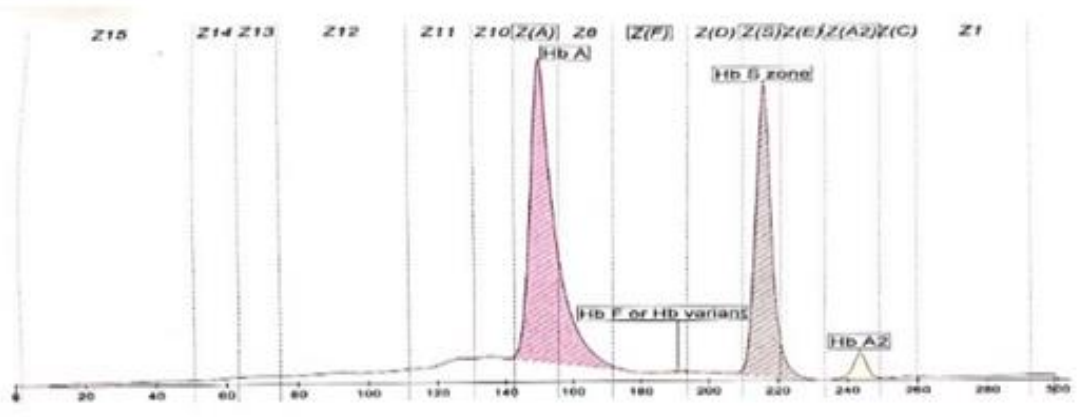


Figure 2-3 Electrophoresis results for a sample of normal haemoglobin.



Haemoglobin Electrophoresis

Name	%	Normal Values %
Hb A	59.7	
Hb F or Hb variant	0.5	
Hb S zone	37.1	
Hb A2	2.7	

Figure 2-4 Electrophoresis results for haemoglobin that carries the sickle cell trait.

2.7 Fatty acid analysis

2.7.1 Introduction

The fatty acid content was analysed at the London Metropolitan University research laboratory. To perform the analysis, the researcher required two weeks of training. The lead supervisor oversaw the researcher's work and his assistant Dr Nada trained the researcher. All safety precautions were taken and the university guidelines were followed. Samples were brought from Sudan in a dry ice package and were kept in a refrigerator at -80°C. Solvents and all required reagents were available. The equipment was kept tidy and worked well. The samples that were analysed were the plasma and RBCs of the two groups of pregnant Sudanese women, those with SCT and those without, both in the first trimester and postnatally. Extraction tubes were labelled carefully with code numbers.

Glassware was preferable to plastic tubes to avoid damage and leak. Plastic tubes may have interfered with the gas-liquid chromatography (GLC) results. Heating during evaporation or nitrogen flushing could have damaged plastic tubes and caused leakage. Plastic tubes contain free radicals, which may have interfered with the GLC results. The solvents that were required were polar methanol (CH_3OH) and non–polar chloroform ($CHCl_3$) organic solvents, normal saline ($NaCl_{(aq)}$), potassium bicarbonate ($KHCO_3$), petroleum ether (CH_3OCH_3), anhydrous sodium sulphate powder (Na_2SO_4), and heptane (C_7H_{16}). Samples were kept in a dark, cool area as much as possible to avoid damage by heat, oxidation, or light effect.

2.7.2 Preparation of solvents and reagents

Most of the solvents contained a measured amount of 2, 6 di-tert-butyl-p-cresol (butylated hydroxytoluene (BHT), this is used to prevent dehydrogenation and oxidation during analysis and storage). Each bottle of chloroform, methanol, petroleum ether, anhydrous methanol and heptane contained 0.25g of BHT. A mixture of chloroform and methanol was frequently used during the analysis. It was prepared by adding chloroform to methanol at a ratio of 2:1 V/V. This mixture was used in the extraction of lipids and the recovery phase. A solution of 0.85% normal saline was prepared by adding 0.85g of sodium chloride to 100ml of purified water. A bottle of 1000ml that contained 8.5g sodium chloride was used in the extraction phase. A solution of 5% normal saline was prepared by adding 5g of sodium chloride to 100ml of purified water (or 12.5g to 250ml) for use in the methylation stage. A solution of 2% potassium bicarbonate was prepared by adding 2.0g of potassium bicarbonate to 100ml of purified water. The most important agent, which required careful preparation, was a solution of 15% acetyl chloride (CH_3COCl) in anhydrous methanol. As described by Christie (1993) and modified by Ghebremeskel *et al*. (2006), 100ml of anhydrous methanol that contained BHT was placed in a dark-brown bottle and kept for 10 minutes at -80°C, after which time the bottle was put in a

bowl of ice. Protective measures (a glass cubicle with gloves and eyeglasses, according to laboratory guidelines) were used while 15ml of acetyl chloride was added drop by drop. The reaction is known to generate heat (exothermic). This reagent was used in the methylation stage when 4ml was added to each sample.

2.7.3 Extraction

The method of Folch *et al.* (1957) was used to extract the lipids, as modified by Ghebremeskel *et al.* (2007). About 15ml of methanol was placed in a 100ml extraction tube. About 500ul of blood, mainly RBCs, was transferred to the extraction tube. The preparation was vortexed for a few minutes and then 30 ml of chloroform was added (Fisher Scientific, Loughborough, UK). Oxygen-free nitrogen (OFN) was flushed through each tube. The samples were kept for 24 hours in a dark freezer at 4°C (Figure2.5).

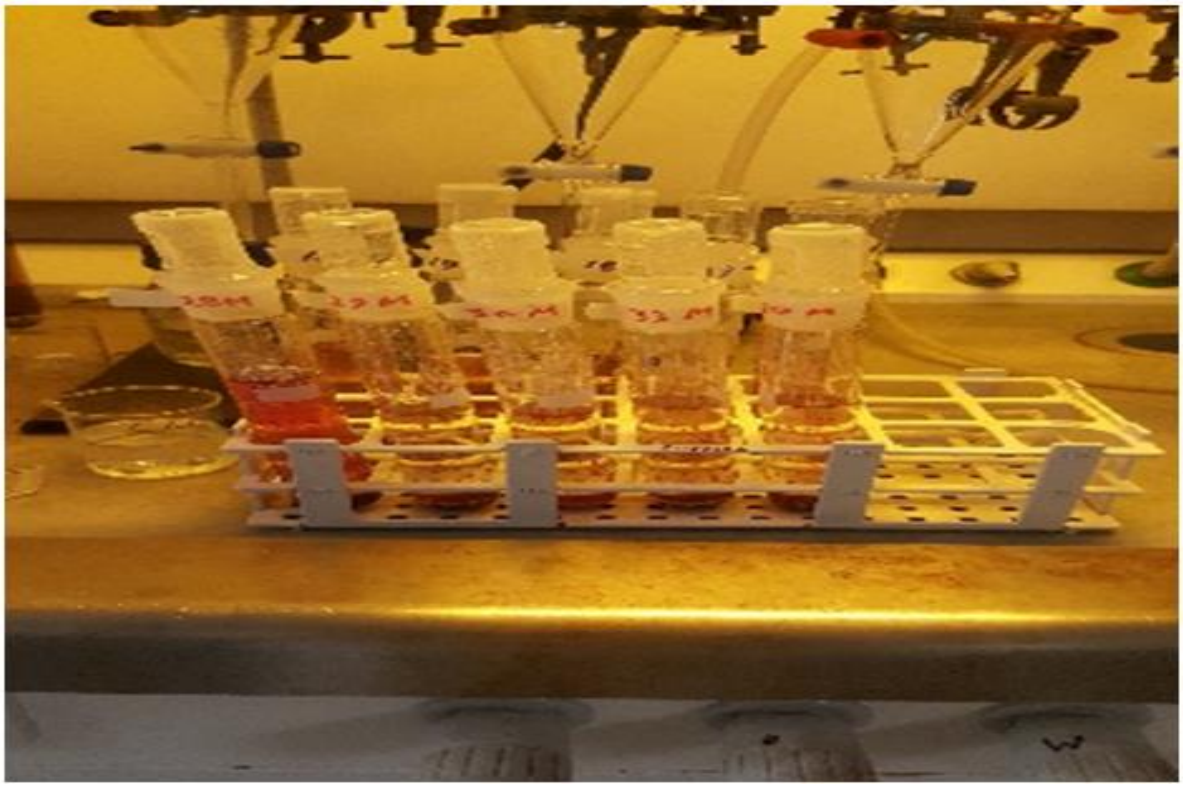


Figure 2-5 Lipid extraction tubes. (Photo taken by Tigani 2020) LNC London Metropolitan University

2.7.4 Separation of lipids

The oval glass separating funnel (Whatman International Ltd., England) was used, topped by a metal funnel and filter paper. The fluid in the extraction tube was transferred to the separating funnel. The label was transferred to the flask bottle. The extraction tube was washed with a mixture of chloroform and methanol (15ml) and about 15ml of physiological saline (0.85%) was added. When the filter paper was dry, OFN was flushed through the funnel. This procedure was liquid-liquid extraction. This preparation was kept at 4°C in a dark place overnight for 24 hours.

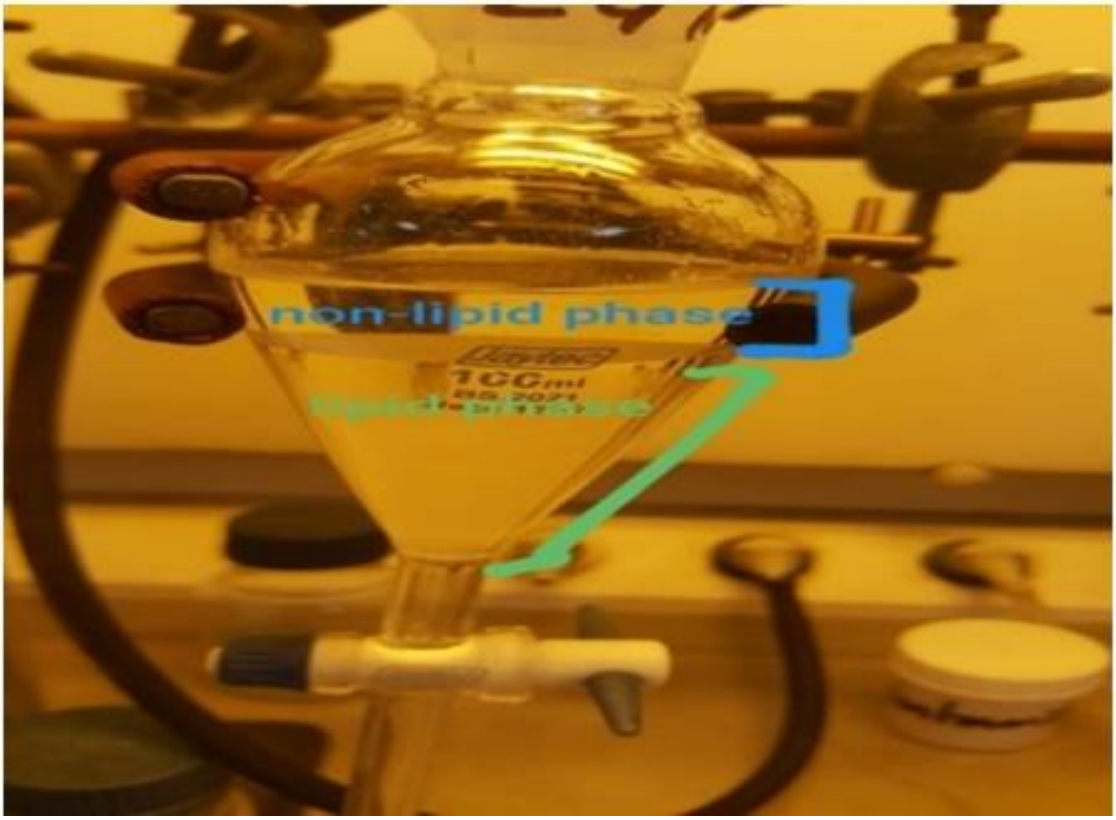


Figure 2-6 Liquid-liquid extraction of lipids: the lower layer in the flask contains lipid (light green), while the upper layer is discarded (blue). Adapted from Nada Abukenisha 2020.

2.7.5 Recovery of total lipids

The next day, the lower layer of lipid was transferred to a round-bottomed glass bottle and the upper layer, which contained non-lipids, was discarded (Figure 2.6).

The collected layer of lipids was placed in a rotary evaporator (Rota Vapor R210 and vacuum pump V-700, Buchi, Switzerland), and the lipids were recovered under vacuum at 37°C. When the bottle was dry, about 2ml of methanol that contained 0.01% BHT was used to clean the neck of the round-bottomed flask and the flask was rotated again until the 2ml had been evaporated. When all the remaining water had been removed by evaporation, the round-bottomed flask looked dry and the fatty acids had adhered to the glass. This thin layer of lipid was recovered by using 6 ml of chloroform/methanol (2:1, V/V) that contained 0.01% BHT. This mixture was transferred to 10 ml methylating vials (Figures 2.7). The vials were flushed with OFN and stored in a dark place at -20°C.

Figure 2-7 Recovery of lipids (left) and transfer of lipids to methylation tube (right) (Photo taken by Nada Abukensha 2020)

2.7.6 Methylation of FAME's

The next day, the vials were warmed to 37°C and OFN was flushed through them until the vials were dry. The vials were taken to a cubicle where the methylating agent was prepared. About 4ml of the methylating agent was added to each tube, which was flushed with OFN, and then vortexed for one minute. Compounds must be stable to achieve high-quality fatty acid analysis results. The fatty acids must be in their methylated form to enable easy conversion into stable but volatile compounds. This conversion reaction is defined as trans-esterification (Christie, 1993). The carboxylic group can react with the hydroxyle group to produce an ester and water (R_1- COOH + R-OH will give R_1-COOR + H_2O). This is a condensation reaction, which is usually reversible if the water is not removed or evaporated. To generate a successful result a reagent is required that will help in this process.

FAMEs were prepared by heating the total lipids in 4ml of the reagent for three hours at 70°C, and after each hour the bottles were vortexed for one minute one after the other. About 4ml of normal saline was placed in each vial to stop the methylation process after the heating period. The next step was to add 2 ml of petroleum ether that contained 0.01% BHT, after which each vial was vortexed for a few minutes. A clear upper layer, which constituted the FAMEs, appeared in the sample (Figure 2.8).

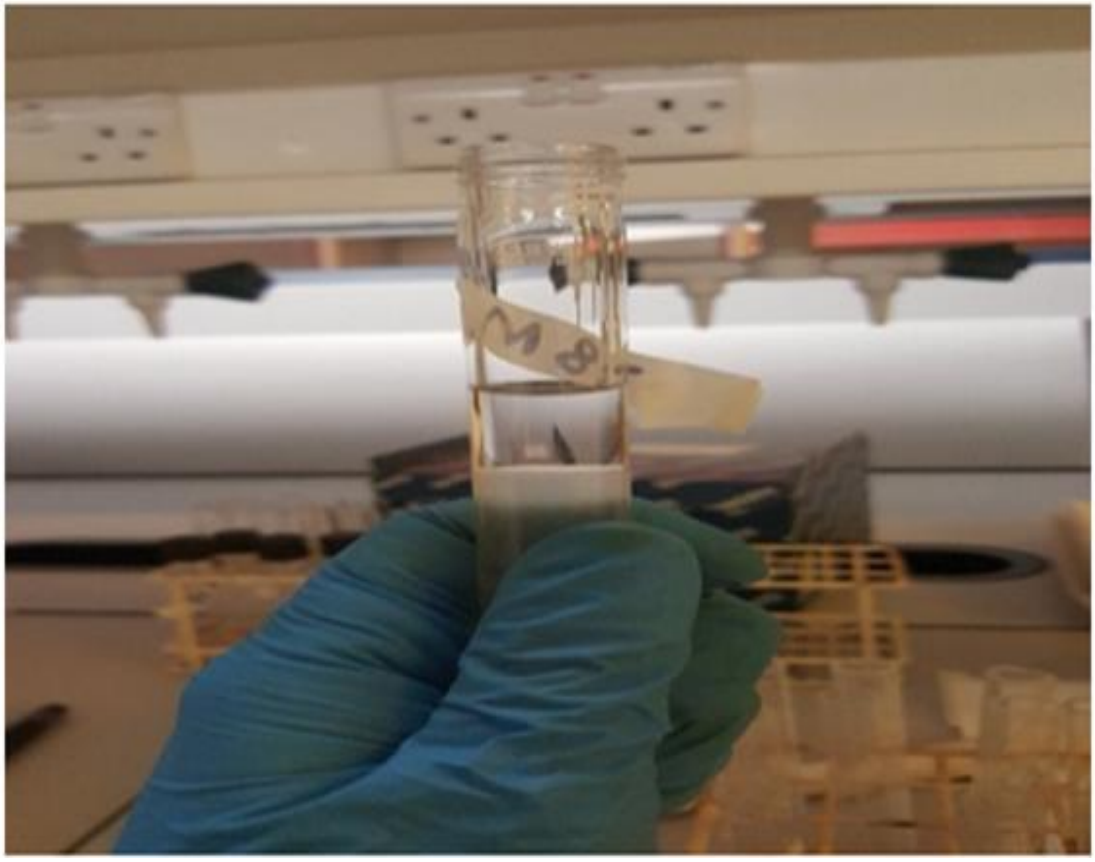


Figure 2-8 clear upper layer in vial indicates presence of FAME's (Photo taken by Tigani 2017)

The FAMEs were transferred carefully into another vial containing 2ml of 2% $KHCO_3$ to reduce acidity in the sample and avoid interference with the GLC procedure. The mixture was vortexed. The above step was repeated and an adequate amount of FAMEs was put in the $KHCO_3$ vial. The upper layer of FAMEs was then transferred to another vial containing 100mg of anhydrous sodium sulphate granules to eliminate any residue of water, the presence of which would otherwise interfere with the GLC procedure. The water-free neutralised FAMEs in petroleum ether were then transferred into small 3ml tubes. The tubes were labelled with both the code number and the identification number. The petroleum ether was then removed by putting the tube under a pool of nitrogen at less than 37°C. When the tubes were dry, 1ml of

heptane (which contained 0.01% BHT) was placed in the tubes and the sample was stored at -20°C.

2.7.7 Analysis of FAMEs by GLC

GLC was used to separate and identify volatile substances and derivatives that were present in the sample. This method involved the use of two phases, a mobile and a stationary phase. The particular method chosen for each sample depended on the features or characteristics of the sample. These features were the physical and chemical state of the sample, molecular weight, polarity, and charge of the ions. Separation was dependent on how long the molecule stayed in the mobile phase. The longer it stayed in the mobile phase, the faster it would flow through the stationary phase.

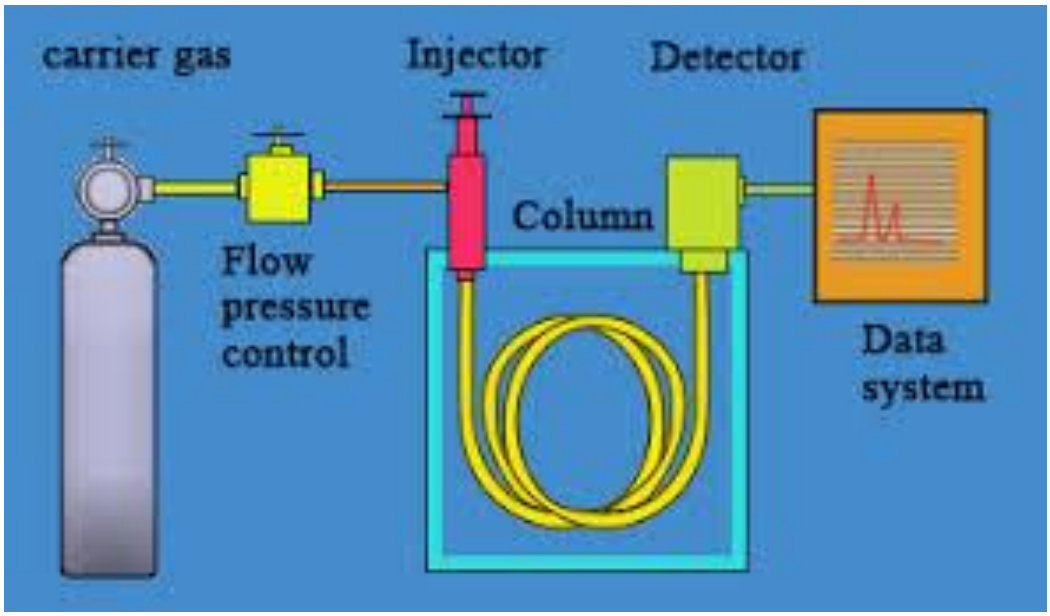
In GLC, an inert gas is used as a one-way-flow mobile phase. This is also known as a carrier gas; it carries the solutes through the stationary phase. As the substance migrates through the stationary phase, the various constituents separate due to their different mobilities. The stationary phase in this methodology was known as the column. Various features must be set to ensure that the GLC machine performs to a high standard. These features are the column length, internal diameter, the thickness of the film, oven temperature, and flow rate of the inert gas.

Table 2-1 Characteristics of GLC

Parameter	Condition
Column Type	BPX-70 Capillary GLC column (30m length x 0.25mm internal diameter x 0.25μm film)
Carrier gas Type Flow rate Hydrogen to detector Pressure	H_2 2.3ml/min 60KPa 6bar
Injection Temperature Volume Type Purge Splitter Split ratio	 240°C 1μl Split-mode 5.6ml/min 41.8ml/min 1:5
Oven Initial temperature	150°C
Detector Type Temperature	Flame – ionisation 280°C
Run time	52 mints

Hydrogen was used as the carrier gas. Other inert gases can be used and the choice depends on the type of column used, cost, safety, the type of detector in use, and the substance being detected. Other features must be considered in the choice of carrier gas, such as the Van Deemer profile. A broad, minimum Van Deemer profile usually provides optimum performance.

Hydrogen is generated instantly during analysis and is used immediately because it is dangerous to keep hydrogen in cylinders. To perform the analysis, we used a low-capacity hydrogen generator that was attached to the GLC machine (the HRGC MEGA 2 series) (Fisons Instruments, Milan, Italy). The machine was equipped with a flame ionisation detector (FID) that could operate at temperatures of up to 280°C. The FID comprised a tip and a collector plate. It had a wide linear range and was stable and sensitive. The signal, which resulted from the potential difference between the FID and the plate, could be amplified and easily transmitted to a computer data station, where it was shown as a peak. If more than one sample was tested at once, more than one peak appeared. Initially, one standard or control sample (heptane+BHT) was passed through. The result was recorded as a model that could be used to detect and monitor contaminants. A sample (1µl) of the FAMEs mixture was injected into the GLC system and the system was left to run for 52 minutes. The peak area retention times were recorded as follows: heptane, 2.8 minutes; BHT, 5.15 minutes. After every 20 samples, one standard sample was injected to ensure that the retention time remained stable and had not shifted. Hydrogen ions were passed through a BPX-70 capillary column (30m long, 0.25mm internal diameter, 0.25µm film). These injections were used as split-mode (Claid Baezzea, Lake Como, Italy). Table 2.2 shows the flow rate, injector temperature, injector purge, and column type. Samples could be analysed in a series of 15 to 20 specimens. The machine could be left to run overnight. Fatty acid content was expressed as a percentage of the total FAMEs. These percentages were quantified through the use of a computer chromatography data system EZ Chrom Elite (v6.6 Scientific Software, and Ramon, CA, USA). The results were copied and transferred to the IBM SPSS Statistics program and analysed to compare the results for the two groups.



Block diagram of gas chromatograph. Adopted from

(https://chemistry.tutorvista.com/analytical-chemistry/gas-chromatography.html

2.8 Quality research techniques

2.8.1 Ethical approvals[1]

Ethical approval was obtained from the FMOH Sudan, the Faculty of Medicine at Khartoum University (Sudan), and the London Metropolitan University (UK). A letter of no objection was received from the Obstetrics and Gynaecology teaching hospital in El-Obeid city, Sudan. The research team consisted of the principal investigator, two coordinators, three senior obstetricians and two laboratory technicians. The fieldwork was undertaken in February-March 2017 in the antenatal clinics of the Obstetrics and Gynaecology teaching hospital.

2.8.2 Consent and invitation letter[2]

An invitation letter was given to each participant to inform them about the study. Informed consent was obtained from participants. Confidentiality of the information was maintained by

[1] Check appendix 1
[2] Check appendices 5 and 6

ensuring that no names were disclosed or written within the questionnaires. Coding was used during the data analysis and in the presentation of the results.

2.8.3 Questionnaire[3]

A questionnaire was used to collect the anthropometric, medical, and obstetrics data and some information about the dietary history of the participants and the types of food that they ate.

2.8.3.1 Structure

The questionnaire was prepared in English and translated into Arabic during the interviews. For the pilot study, the questionnaire was pre-tested in El-Obeid Teaching Hospital, Western Sudan. This procedure was carried out before the required data was collected to help with the accuracy of responses and to estimate the time required to complete the questionnaire. During the pilot study, some questions were rephrased to ensure that the interviewer provided valid information. The women who participated in the pilot study were excluded from the main survey. Trained persons completed the questionnaires and the same procedure was used in the main study. The questionnaire answers were coded by the researcher to ensure reliability. A questionnaire is an appropriate instrument for the collection of data that is beyond physical reach (Leedy, 1993). The use of a questionnaire enables the collection of large amounts of information from a large number of people in a short period, in a relatively cost-effective way, with limited effect on its validity and reliability. The results of the questionnaires can be quantified easily and quickly by a researcher and can be analysed more objectively than data gathered through other research methods (de Barros *et al.,* 2020). However, answers to questionnaires may not provide an understanding of how or why people act as they do, and to gain this information, complementary qualitative methods may be required to generate in-depth

[3] Check appendices 7 and 8

data. Sometimes it is difficult to understand some information that is provided through a questionnaire, or the respondent may have forgotten or does not think within the full context of the situation during the completion of the questionnaire. Respondents may understand questions differently and therefore reply based on their interpretations of the questions (de Barros *et al.*, 2020).

2.8.3.2 Validity

Validity and reliability are instruments that measure the consistency and accuracy of a survey. Validity is defined as the degree to which the outcome results measure the matter that one wishes to measure (de Vet *et al.*, 2011). Reliability refers to the ability to gain the same results during repeat performances of the survey. The reliability is tested through the administration of the survey among a group of respondents, then repeating the survey with the same group at a later point in time. The responses at the two-time points are compared (Leedy and Ormrod, 2005).

2.9 Statistical analysis

Quantitative data were tested for normality and homogeneity of variance and subsequently analysed through the application of an independent t-test (parametric data). When the data was not normally distributed, the Mann-Whitney U test (non-parametric data) was used .The data were expressed as mean ± SD or as percentages.

Qualitative or categorical data (Socio-demographic, clinical and laboratory data) with cell frequency of five or more were assessed through the application of a Chi-square test.

The data were analysed through the use of IBM SPSS Statistics for Windows, version 26 (IBM SPSS Ltd., Woking, Surrey, UK). The significance level was set at $p<0.05$, as shown in chapters 3, 4 and 5.

Chapter 3

3. Nutritional and haematological status of Sudanese women of childbearing age with steady-state Sickle cell Anaemia (SCA).

3.1 Introduction

Sickle cell anaemia is inherited by a single base substitution at codon 6 of the β-gene for haemoglobin. However, there is significant clinical heterogeneity of the disease, which is probably attributable to genetic, epigenetic, nutritional and environmental factors that interact with each other (Rahimy *et al.,* 2003). Generally, care for patients with SCA has improved due to earlier diagnosis than was previously the case, the widespread use of penicillin prophylaxis, vaccination, folate supplementation, and access to comprehensive care programmes that include blood transfusions and hydroxyurea therapy. These changes have significantly impacted morbidity and mortality rates (Adamkiewicz *et al.,* 2008; Elderdery *et al.,* 2011; Gaston *et al.,* 1986; Rees *et al.,* 2010; Vichinsky, 1991).

Nutritional status and growth and development are important indicators of the overall health and well-being of patients with SCA. Patients with sub-optimal nutrition status are at increased risk of hospital admission, severe morbidity and early mortality. The health of people with SCA is often compromised by a high metabolic rate, reduced absorption of essential nutrients and a loss of appetite, all of which are induced by the disease and its treatment. Also, a lack of access to nutrient-dense foods is a major problem for sickle cell patients in economically disadvantaged countries and communities.

It is widely recognised that maternal nutritional status before pregnancy is one of the main determinant factors of pregnancy outcome (Johnson *et al.,* 2017; Mavalankar *et al.,* 1994; Pan

et al., 2016; Patel *et al.,* 2018; Vobecky, 1986; Young *et al.,* 2015). Published data are scarce on the nutritional status of women of childbearing age with SCA. Indeed, the effect of nutritional status before pregnancy on maternal and foetal outcomes has not been studied in women with the disease.

Until recently, it was rare to find pregnant women with SCD in Sudan. However, because of improved disease management and the resulting increase in life expectancy, their number has increased significantly. Regardless, the country is highly underdeveloped with rudimentary health services and rampant poverty and malnutrition. In this highly patriarchal society, women, young children and individuals with chronic diseases tend to bear the brunt of gross malnutrition and under-nutrition.

3.2 Objectives of this study

The aim was to investigate the nutritional status and health of Sudanese women of childbearing age who had SCA. Anthropometry and haematology were used to assess their nutritional status and their health and disease conditions, respectively.

3.2.1 Subjects

Women with steady-state SCA (HbSS, n=39) and without (HbAA, n=36) were recruited during a routine visit to the haematology clinic at the University of Khartoum Ibn Auf Teaching Hospital. The inclusion criteria were: non-pregnant women aged 16-40 years who had volunteered to participate in the study and who had the mental capacity to give informed consent. The exclusion criteria were: anyone in sickle cell crisis, who had suffered acute illness or received a blood transfusion in the previous four months, who had other chronic diseases or a physical disability that impaired their access to food or restricted their eating, and pregnancy. Steady-state was defined as an absence of sickle cell crisis or acute illness from four months before, and up to two weeks after, the collection of blood for the study. Blood specimens (5ml)

and detailed anthropometric and demographic data and information on medical history and dietary habits were collected. Ethical approval was obtained from the FMOH of Sudan, the University Of Khartoum Medical School and the London Metropolitan University, and informed and signed consent was gained from the participants.

3.2.2 Methods

Demographic data was collected from the patients with their medical history from hospital records and the patients. These data were collected with the use of a questionnaire that had been developed for the study. Anthropometric measurements - weight in kilograms and height in centimetres - were taken through the use of a Seca Electronic Scale 890 (UNISCALE, Seca, Birmingham, UK) and a height measuring board (Shorr, Olney, Maryland, USA), respectively. Haematological variables (haemoglobin concentration in g/l, PCV, MCV, MCH, TWBC, and PLTs) were measured with the use of the Sysmex KX-21 N automated haematology analyser (Sysmex Corporation, Kobe, Japan).

3.3 Data analysis

Data are expressed as mean ± standard deviation (SD) or percentages. Quantitative data were tested for normality and homogeneity of variance and subsequently analysed through the use of an independent t-test (parametric data) or a Man-Whitney U test (non-parametric data). Categorical (Qualitative) data (socio-demographic, clinical and laboratory characteristics) with cell frequency of five or more were assessed through the application of a Chi-square test on the contingency platform. When the observed cell count was fewer than five, the Chi-square, Yate's correction of continuity and Fisher's exact tests were used under the assumption of independence of rows and columns and conditional on the marginal totals. The significance level was set at $p<0.05$. The data were analysed through the use of IBM SPSS Statistics for Windows, version 26 (IBM SPSS Ltd., Woking, Surrey, UK).

3.4 Results

Table 0-1 Mean age (± SD) and clinical parameters of the participants

	HbSS (n=39)	**HbAA (n=36)**	**p-value**
Age (years)	19.0 ± 2.7	19.8 ± 2.7	NS
Weight (kg)	39.0 ± 8.9	53.8 ± 11.0	0.001
Height(cm)	151.0 ± 11.7	158.0 ± 6.7	0.002
Body mass index (BMI) (kg/m^2)	17.2 ± 4.3	21.5 ± 4.2	0.001

NS = not significant; SD = standard deviation

Table 0-2 Demographic and clinical variables of the participants

		HbSS (n=39)	**HbAA (n=36)**	**p-value**
BMI (kg/m^2)	Categories	n (%)	n (%)	p-value
	Underweight (<18.5kg/m²)	27 (69.2)	9 (25.0)	0.001
	Normal weight (18.5-24.9kg/m²)	11 (28.2)	20 (28.2)	0.000
	Overweight / obese(>25kg/m²)	1 (2.6)	7 (19.4)	0.209
Education	*Illiterate & primary school*	24 (61.5)	3 (8.3)	0.000
	Middle & high school	13 (33.3)	11 (30.5)	
	University	2 (5.1)	22 (61.1)	0.000
Parental relationship	*1st & 2nd-degree relatives*	25 (64.1)	16 (44.4)	0.037
	Unrelated	13 (33.3)	20 (55.6)	0.001

Sibling with SCD	*Yes*	22 (56.4)	3 (8.3)	0.000
	No	17 (43.6)	33 (91.7)	0.000

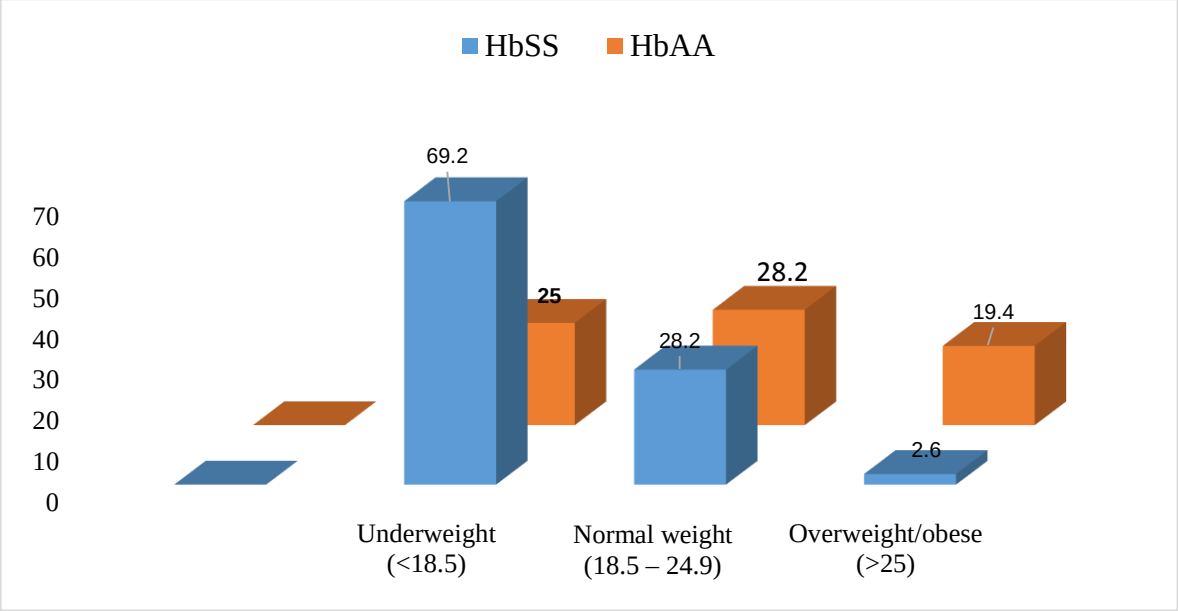


Figure 0-1 Two groups of women with and without SCA are categorised according to BMI.

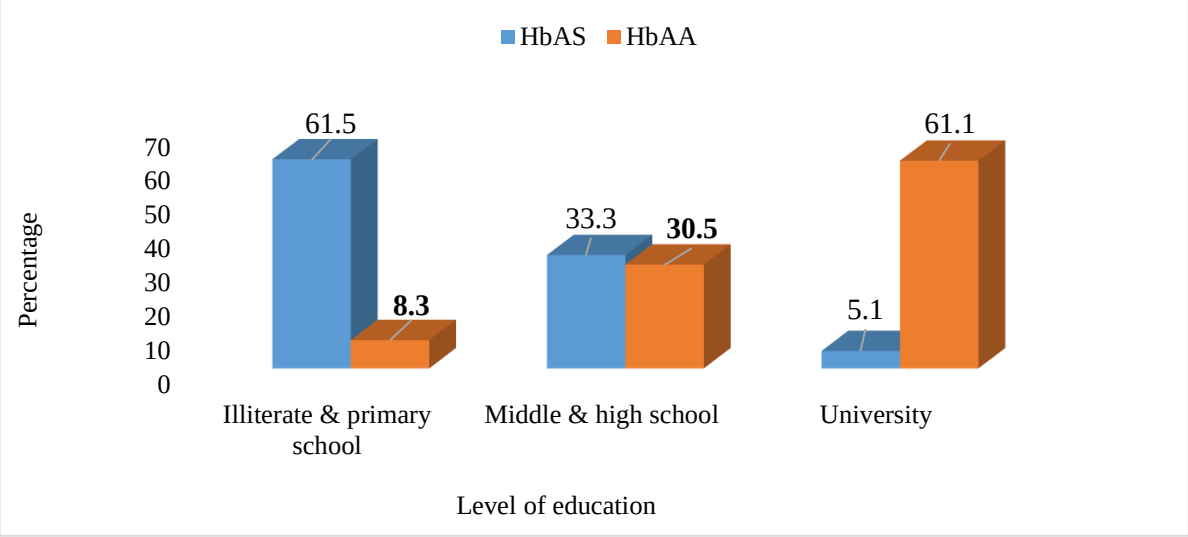


Figure 0-2 Education levels for the two groups as percentages.

Table 0-3 Daily food consumption of the participants

	Response	**HbSS (n=39), n (%)**	**HbAA (n=36), n (%)**	**p-value**
Regular breakfast	Yes	37 (94.9)	34 (94.4)	p>0.05 (NS)
	No	2 (5.1)	2 (5.6)	
Regular lunch	Yes	36 (92.3)	34 (94.4)	NS
	No	3 (7.7)	2 (5.6)	
Regular dinner	Yes	23 (59.0)	14 (38.9)	NS
	No	16 (41.0)	22 (61.1)	

NS=not significant

Table 0-4 Haematological characteristics of the participants

	HbSS (n=39) (mean±SD)	**HbAA (n=36) (mean±SD)**	**p-value**
Haemoglobin (g/dl)	9.2 ± 1.6	14.3 ± 1.6	0.000
PCV (%)	25.1 ± 6.2	37.6 ± 3.3	0.000
MCV (femtolitres, fl)	88.9 ± 13.5	81.4 ± 5.7	0.003
MCH (pg)	34.5 ± 3.6	31.0 ± 3.0	0.000
TWBC (x 10^3) µl	11.4 ± 4.2	5.9 ± 2.1	0.000
Neutrophils (%)	51.6 ± 11.3	47.9 ± 9.9	NS
Lymphocytes (%)	38.8 ± 9.1	41.0 ± 9.5	NS
PLTs (x 10^3) µl	409.1 ± 121.5	328.5 ± 95.6	0.002
Systolic blood pressure (BP)	109.5 ± 10.4	108.5 ± 9.6	NS

Diastolic BP		67.4 ± 8.4	70.4 ± 8.3	NS
		n (%)	**n (%)**	
Blood transfusion history	YES	32 (82.1)	1 (2.9)	<0.001
	NO	7 (17.9)	33 (97.1)	
Folic acid supplement	YES NO	38 (97.4) 7 (17.9)	1 (2.9) 1 (2.9)	<0.001
Hydroxyurea use	YES NO	25 (64.1) 14 (35.9)	0 (0) 0 (0)	<0.001
Omega-3 fatty acid supplement	YES NO	4 (10.3) % 35 (89.78)	0 (0) 0 (0)	NS

NS = not significant; SD = standard deviation

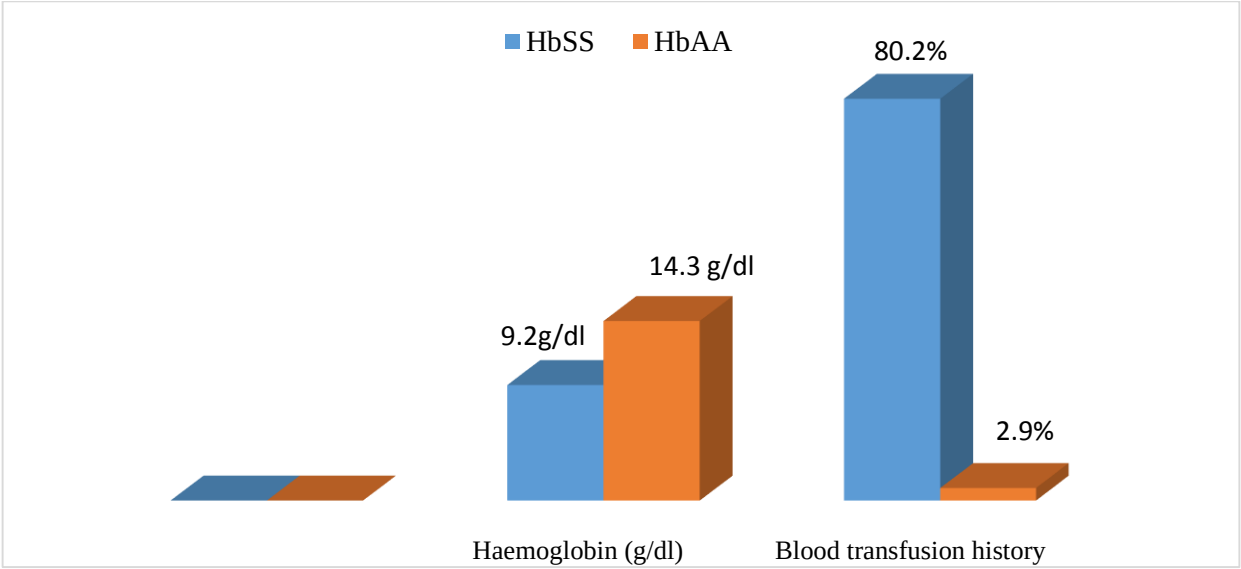


Figure 0-3 Haemoglobin levels (g/dl) and history of blood transfusion (%) for the two groups of women under study.

Table 0-5 Anthropometric and haematological characteristics of the women with SCA who had been treated or not treated with hydroxyurea.

	Treated (n=25)(mean ± SD)	**Untreated (n=14) (mean ± SD)**	**p-value**
Age	18.9 ± 2.4	19.3 ± 3.1	NS
Weight	39.7 ± 9.7	37.7 ± 7.4	NS
Height	151.4 ± 13.2	150.3 ± 8.5	NS
BMI	17.50 ± 5.0	16.6 ± 2.5	NS
Systolic BP	109.0 ± 10.4	110.4 ± 10.5	NS
Diastolic BP	65.4 ± 8.2	71.1 ± 7.9	NS
Haemoglobin (g/dl)	9.2 ± 1.8	9.2 ± 1.1	NS
PCV (%)	25.0 ± 7.3	25.2 ± 3.8	NS
MCV (fl)	92.0 ± 6.3	83.4 ± 2.2	NS
MCH (pg)	35.2 ± 3.5	33.2 ± 3.6	NS
TWBC (x 10^3) µl	11.8 ± 4.2	10.7 ± 4.2	NS
Neutrophils (%)	51.4 ± 10.4	52.0 ± 13.2	NS
Lymphocytes (%)	37.7 ± 8.4	40.6 ± 10.5	NS
PLTs (x 10^3) µl	412.2 ± 130.2	403 ± 108.8	NS
	N (%)	**n (%)**	
History of blood transfusion: YES	20 (80)	12 (85.7)	NS
History of blood transfusion: NO	5 (20)	2 (14.2)	NS

Folic acid supplement: YES	25 (100)	13 (92.8)	NS
Folic acid supplement: NO	0 (0)	1 (7.2)	NS

NS = not significant; SD = standard deviation

3.5 Analysis of results and comments

3.5.1 HbSS versus HbAA

The anthropometric and demographic characteristics of the women with SCA (HbSS) and without (HbAA) are presented in Tables 3.1 and 3.2. There was no difference in the mean age between the HbSS and HbAA groups ($p>0.05$). However, the latter group were taller ($p=0.002$), heavier ($p=0.000$) and had a higher mean BMI compared with the former (Figure 3.1). The HbAA women had higher rates of literacy and attendance in tertiary education ($p=0.000$) (Figure 3.2).

The daily food consumption of the participants is shown in Table 3.3. Most of the participants in the study ate regular breakfast (94.9% SCA vs. 94.4% healthy) and lunch (92.3% SCA vs. 94.4% healthy). However, significant proportions of both groups did not consume dinner regularly (HbSS, 59% vs. HbAA, 61.1%, $p<0.05$) due to financial constraints.

Clinical findings: four members of the HbSS group showed enlarged livers (by 4cm, n=2; 2cm, n=2) and one had an enlarged spleen (by 6cm). None of the patients had a scar that would indicate splenectomy. A few of the HbSS patients had histories of painful crises.

Haematology: The haematological data for the two groups of women are presented in Tables 3.4 and Figure 3.3. The women with SCA had lower PCV and haemoglobin concentration ($p=0.000$) and higher MCHC ($p=0.000$), MCV ($p=0.003$), TWBC ($p=0.000$) and PLT counts ($p=0.003$) compared with the healthy controls. There was no difference in lymphocyte or

neutrophil counts, or in systolic and diastolic blood pressures (BPs), between the two groups ($p>0.05$).

3.5.2 HbSS (hydroxyurea-treated and untreated)

Of the 39 patients with SCA who consented to participate in the study, 25 were on hydroxyurea treatment. Anthropometric, demographic and haematological data for those treated with hydroxyurea and those who were not treated, at a steady state, are presented in Table 3.4.

There were no differences in age, weight, height, BMI and systolic and diastolic BPs between the treated and untreated groups ($p>0.05$). The hydroxyurea-treated and untreated patients had similar histories of blood transfusion rates, comparable levels of haemoglobin concentration, PCV and MCHC, and counts of TWBCs, PLTs, neutrophils and lymphocytes ($p>0.05$). The percentages of patients who were receiving folic acid supplementation were not significantly different (treated 100%, untreated 92.8%, $p>0.05$). The only difference observed was in the MCV, which was higher in the hydroxyurea-treated patient group.

3.6 Discussion

Consanguineous matrimony, or marriage between first and second cousins, is a common tradition in all Sudanese tribes. This is evident from the high proportion of parental blood relationships that were reported among the members of the HbSS (64%) and HbAA (44%) groups. Consistent with our findings, Daak *et al.* (2016) reported a high rate of parental and self-consanguineous marriages in patients with SCD in Western Kordofan State, Sudan.

In this study, although a significant number of the controls and patients were born to parents who were blood relatives, the figure was more remarkable in the group that had SCA. It may be that the social stigma associated with the disease makes it very difficult for affected individuals to find a husband or wife from outside the immediate family circle. Regardless, as

is apparent from the number of patients who reported having siblings with SCA, consanguinity/inbreeding can be expected to perpetuate and indeed increase the prevalence of the disease in Sudan.

There is an urgent need for comprehensive and well-planned education, social awareness and counselling programmes targeted at patients with SCA and communities, particularly those in rural areas with deep-rooted traditions, to help to reduce discrimination and stigma. It has been reported, in Sudan, that the strong predictors of negative attitudes and discrimination against SCD are low levels of knowledge and low socioeconomic status (Daak *et al.,* 2016). Similar observations have been reported from other sub-Saharan African countries (Marsh *et al.,* 2011; Tusuubira *et al.,* 2018).

Illiteracy is almost the norm among patients with SCD and very few complete high school or gain a university education. In the current study, the patients without any formal education (illiterate) and those who attended primary school accounted for 61.5% of the SCA group, and only two SCA patients (5.1%) had achieved tertiary education (Figure 3.2). Compared with their age- and gender-matched classmates, an appreciable number of children with the disease have been reported to underperform drastically in school (Armstrong *et al.,* 1996; Ogunfowora *et al.,* 2005; Akenzua and Olanrewaju, 1995), due to disease-caused absences from school, neurological abnormalities that result from silent or overt strokes, poverty and psychological and psychiatric issues (Crosby *et al.,* 2015; Ezenwosu *et al.,* 2013; Smith *et al.,* 2013). Approximately 70% of the HbSS group appeared to be underweight, while only 25% of the healthy group appeared to be underweight (Figure 3.1).

This study being original and rare in the region conducted to show the effect of the disease in the health of women at child bearing age. The criteria of selection, the data collected through

the questionnaire, the anthropometric measures taken were all performed in a calm and quiet atmosphere, the investigator was aware of each and every detail information. The blood analysis was done same time of collection and some results were handed over to the participants.

There were no differences in the mean age, ethnicity and frequency of daily food consumption between the two groups of women. However, the SCA group had lower weight, height and BMI than the control group of healthy women. These findings are consistent with the results of previous studies (Ebomoyi *et al.,* 1989; Kumar, 2017; Lukusa *et al.,* 2017; Patel *et al.,* 2018; Sadarangani *et al.,* 2009; Telfer *et al.,* 2007; Um *et al.,* 2019; Vobecky, 1986; Young *et al.,* 2015).

In contrast to our findings, high BMI and levels of obesity have been reported in children and adults in the USA who have SCA (Chawla *et al.,* 2013; Farooqui *et al.,* 2014). Therefore, having the disease is not a bar to gaining weight, and it appears that the anthropometric deficits that are commonly observed in patients with the disease in developing countries could be rectified through the provision of appropriate nutritional care and clinical management, particularly during childhood and adolescence.

The sickle cell patients who participated in this study were in a steady state and some of them stated that they took hydroxyurea (35%), folic acid (82%) and omega-3 fatty acid supplements (12.8%). However, their haematological profiles, which were abnormal, were significantly different from those of the healthy control women. This finding was unexpected since hydroxyurea is known to rectify haematological abnormalities in SCD (Charache *et al.,* 1995; Daak *et al.,* 2011; Nevitt *et al.,* 2017; Rigano *et al.,* 2018; Wang *et al.,* 2011). Nevertheless, it was observed that those who took hydroxyurea were heavier, taller and had greater BMIs than

those who did not take it. The hydroxyurea-treated group also had a higher mean MCV value than those who were not taking hydroxyurea.

It is plausible that the patients might not have been taking hydroxyurea as prescribed by their physicians because of its side effects (which include constipation/diarrhoea, hair loss, and muscle and joint pain), financial difficulties (patients in Sudan must pay for medication), an inability to understand prescription guidelines, and/or the drug became unavailable in pharmacies.

Indeed, the haematological data for the hydroxyurea-treated and untreated patients revealed that it was unlikely that the patients were taking the medication as per guidance by the pharmacist/doctor. This is because the data were similar for the non-treated and treated patients. Since most sickle cell patients in the country are very poor, the government should explore the possibility of partially or wholly subsidising hydroxyurea. Also, doctors must check regularly that their patients are following the prescription guidelines.

The study had several limitations: it involved a small sample size (more patients who fulfilled the inclusion and exclusion criteria could not be found in the same clinic); no data were collected regarding quantitative nutrient intake (because many of the participants were illiterate and therefore unable to record dietary intake reliably), and HbF tests were not performed (lack of the relevant laboratory facility). Regardless of these limitations, the study has provided a good picture of the nutritional and health status of Sudanese women of childbearing age who have SCA.

3.7 Conclusion

The low anthropometric measures (height, weight and BMI) and abnormal haematological values of the women with SCA in a steady state were a reflection of sustained nutrition insults that were inflicted by the disease and poverty. Tailored nutritional counselling/advice must be an integral part of the management of patients with SCA. Such advice is vital, particularly for women, because of the adverse effects of pre-pregnancy nutritional deficiencies on birth outcomes.

Chapter 4

4. Sickle cell trait is associated with adverse outcomes of pregnancy in Sudanese women

4.1 Introduction

Sickle cell disease is a group of genetic blood disorders characterised by a mutation involving haemoglobin's β-chain. The inheritance of two abnormal genes, one from each parent gives rise to SCA (HbSS) and one abnormal gene from one parent gives rise to the heterozygous state of SCT (HbAS) (Serjeant, 2001).

Archibald (1926) was the first person to report the presence of the HbS gene in Sudan. Subsequently, several studies revealed the country had a high prevalence of SCD, with an HbS allele frequency that ranged between 0.8% in the north to over 30% in the western part of the country (Adam *et al.*, 2019; Daak *et al.*, 2016; El-Hazmi *et al.*, 2011).

The high HbS allele frequency is due to consanguineous marriages, an influx of tribes affected by the disease from West Africa and a history of endemic malaria (Sabahelzain and Hamamy, 2014; Saha *et al.*, 1990).

Sickle cell anaemia is associated with severe haematological and clinical manifestations, including recurrent vaso-occlusive crises, anaemia, poor neurological, renal and hepatic growth, ophthalmological complications (Claster and Vichinsky, 2003) and poor pregnancy outcomes (Barfield *et al.*, 2010). In comparison, individuals with SCT generally do not display the haematological and clinical symptoms of SCA. Indeed, some of them are not even aware that they carry the faulty gene. However, there is evidence that they exhibit SCA

complications during stressful situations or life events (Goodman *et al.,* 2014; Naik and Haywood, 2015; Tsaras *et al.,* 2009) or vigorous physical activities (Mitchell, 2018).

Pregnancy is a stressful physiological event that is often associated with emotional changes, anxiety and depression (Bjelic, 2018; Weerth and Buitelaar, 2005). Findings on

the effect of SCT on pregnancy outcomes have been equivocal. Some studies have reported adverse outcomes (Hamdi et al., 2002; Hoff et al., 1983; Kose and Kose, 2019; Manzar, 2000; O'Hara et al., 2020; Rathod et al., 2007; Taylor et al., 2006; Ugboma and George, 2015; Wilson et al., 2020), whereas others have not (Abdulsalam et al., 2003 Adeyemi et al., 2009; Baill and Witter, 1990; Tuck et al., 1983; Wellenstein et al., 2019).

The reasons why the findings are contradictory are not clear. However, factors such as nutritional status before and during pregnancy may play a significant role. Symptoms such as nausea and vomiting can lead to dehydration and weakness. Although the prevalence of sickle cell genes in Sudan (Daak et al., 2016; Munsoor and Alabid, 2011) and other sub-Saharan African countries (Piel et al., 2010) is high, published data are scarce on pregnancy outcomes in women with SCT.

4.2 Aim of the study

The aim was to investigate pregnancy outcomes in Sudanese women who had sickle cell trait.

4.2.1 Subjects and methods

4.2.1.1 Subjects

A group of 367 pregnant women who were attending their first antenatal appointment at El-Obeid Hospital, Kordofan, Western Sudan, during their first trimester, and who consented to participate in the study, were screened for SCT. Of the 367 women, 34 had SCT (HbAS), and the remaining 333 had normal haemoglobin (HbAA). The 34 women with SCT and 60 of the

HbAA women who fulfilled the inclusion criteria were recruited to participate in the study. The inclusion criteria were: women aged between 18 and 40 years, who planned to give birth in the same area without moving away, and who were mentally competent to give informed consent. The exclusion criteria were: the presence of SCA, thalassaemia or other chronic diseases, or a physical disability that restricted the person's access to food or ability to eat, and those with malnutrition (223 pulled out after the screening stage, and 50 were excluded according to the criteria).

Detailed demographic, obstetric, medical history, dietary habit and birth outcome data were meticulously documented. A blood sample (5ml) was collected from each woman at enrolment and at delivery. Ethical approval for the study was obtained from the FMOH of Sudan, the University of Khartoum Medical School and the London Metropolitan University, and the participants gave informed and signed consent.

4.2.1.2 Methods

Regarding demographics, a questionnaire that had been developed for the study was used to extract obstetric, medical and haematological history data.

Each woman's weight and height were assessed with a Seca Electronic Scale 890 (UNISCALE, Seca, Birmingham, UK) and a height measuring board (Shorr, Olney, Maryland, USA), respectively.

Regarding blood variables, the Sysmex automated haematology analyser (Sysmex Corporation, Kobe, Japan) was used to measure haemoglobin concentration, PCV, MCV, MCH and TWBC and PLT counts. The level of HbS in each woman's blood was quantified through the use of an electrophoresis machine (Minicap Sebia Flex Piercing, Lisses, France). Details of the method are given in Chapter 2, the methodology section.

4.2.2 Statistical analysis

Quantitative data were tested for normality and homogeneity of variance and subsequently analysed through the application of an independent t-test (parametric data). When the data was not normally distributed, the Mann-Whitney U test (non-parametric data) was used .The data were expressed as mean ± SD or as percentages, and the level of statistical significance was set at $p<0.05$.

Qualitative or categorical data (Socio-demographic, clinical and laboratory data) with cell frequency of five or more were assessed through the application of a Chi-square test on the contingency platform. Chi-square, Yate's correction of continuity and Fisher's exact tests were used when the observed cell count was less than five, under the assumption of independence of rows and columns and conditional on the marginal totals. The SPSS Statistics program for Windows, version 26 (IBM SPSS Ltd., Woking, Surrey, UK) was used to analyse the data.

4.2.3 Results

Table 4-1 Demographic and clinical characteristics of the women with SCT (HbAS, n=34) and without (HbAA, n=60) at baseline.

		HbAS (mean±SD)	**HbAA(mean±SD)**	**p-value**
Age (years)		26.5 ± 6.0	27.2 ± 6.5	.639
Weight (kg)		63.4 ± 15.1	61.4 ± 13.1	.500
Height (cm)		160.8 ± 6.1	157.6 ± 9.7	.081
BMI (kg/m^2)		24.3 ± 4.8	24.9 ± 6.0	.645
Bloodglucose (mmol)		5.1 ± 0.6	5.6 ± 1.5	.117
		n (%)	**n (%)**	

Education	Illiterate & primary	15 (44.1)	15 (25.0)	.108
	Middle & high school	16 (47.1)	33 (55.0)	
	University	3 (8.8)	12 (20.0)	0.000
Occupation	Employed	4 (11.8)	7 (11.7)	.989
	Unemployed	30 (88.2)	53 (88.3)	
Parental relationship	1st & 2nd degree	21 (61.8)	34 (56.7)	.630
	Unrelated	13 (38.2)	26 (43.3)	
Spouse relationship	1st & 2nd degree	22 (64.7)	26 (43.3)	.046
	Unrelated	12 (35.3)	34 (56.7)	
Siblings with SCD	YES	22 (64.7)	8 (13.3)	.000

SD = standard deviation

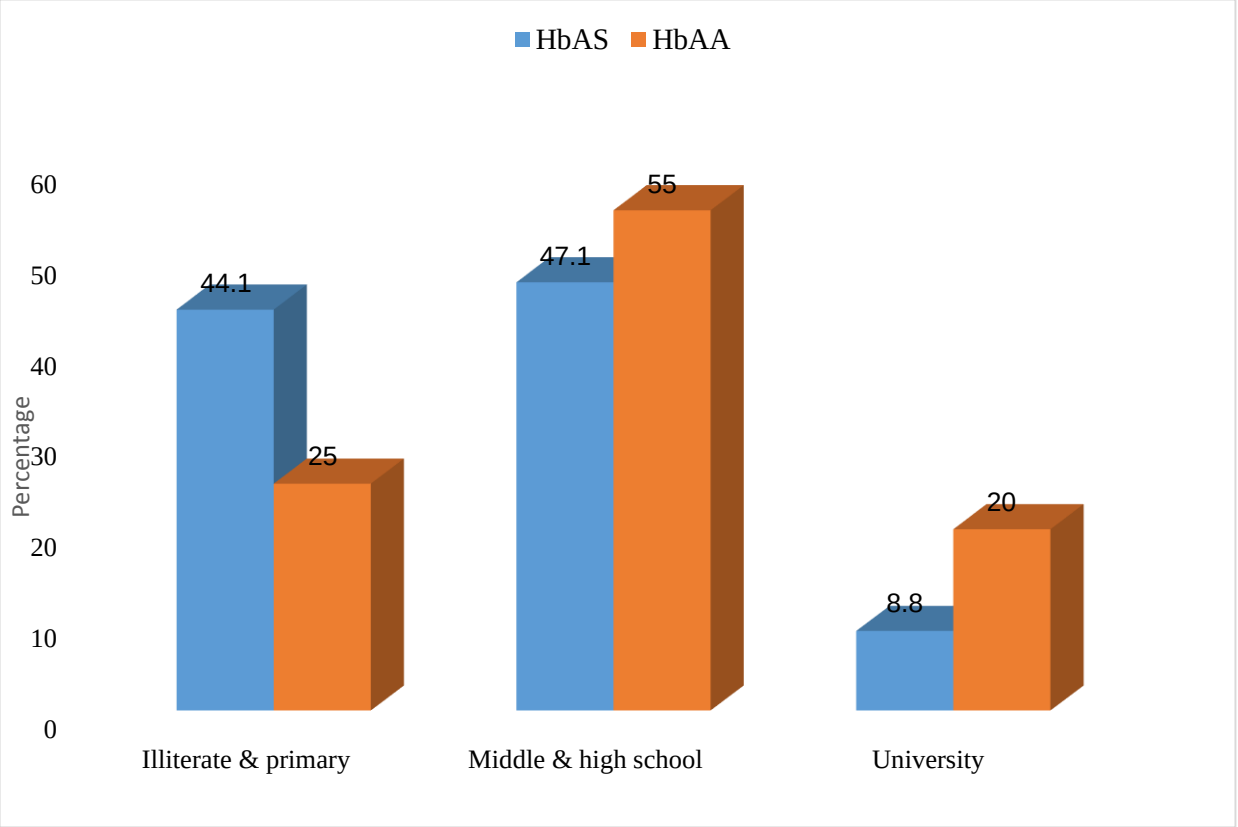


Figure 4-1 Education levels of HbAS and HbAA pregnant women.

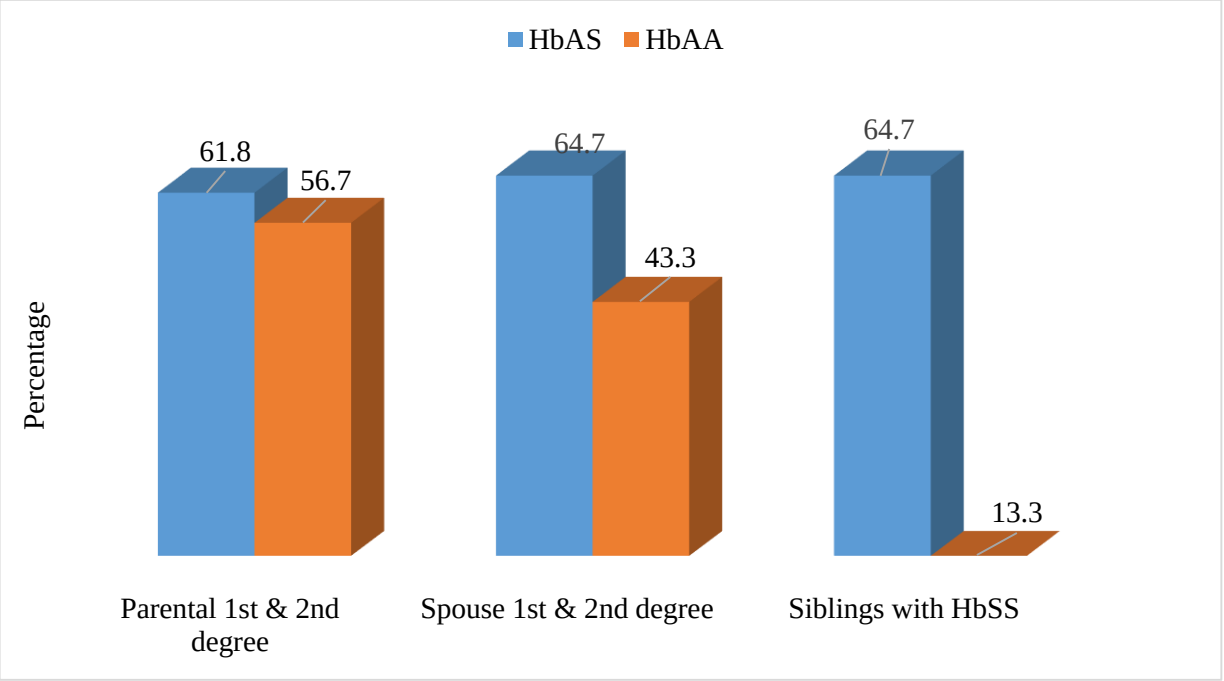


Figure 4-2 Proportions of consanguineous marriages and relationships among pregnant women with SCT and without.

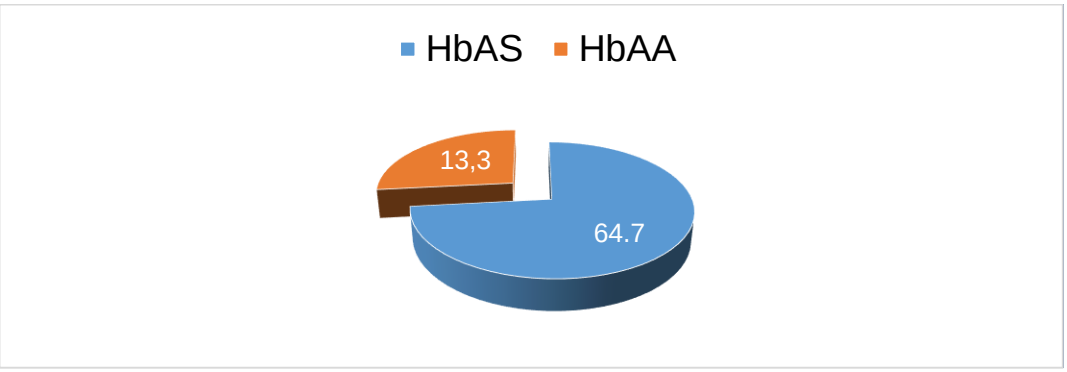


Figure 4-3 Proportion of women with and without SCT who had siblings with SCA.

Table 4-2 Meal frequency, house ownership and income data in Sudanese pounds of the pregnant women with SCT (HbAS, n=34) and without (HbAA, n=60) at enrolment

Meals	Response	HbAS, N (%)	HbAA N (%)	P-value
Have regular breakfast	Yes	33 (97.1)	58 (96.7)	.917
	No	1 (2.9)	2 (3.3)	-
Have regular lunch	Yes	33 (97.1)	59 (98.3)	.681
	No	1 (2.9)	1 (1.7)	-
Have regular dinner	Yes	23 (67.6)	49 (81.7)	.123
	No	11 (32.4)	11 (18.3)	-
Household income	Low <1,000	21 (61.8)	36 (60.0)	.297
	Average 1,000–2,000	13 (34.2)	20 (30)	-
	High >2,000	0 (0%)	4 (6.7)	=
House ownership	Own house	14 (41.2)	36 (60.0)	.210
	Rent	7 (20.6)	9 (15.0)	-

	Live with relatives	13 (38.2)	15 (25.0)	-

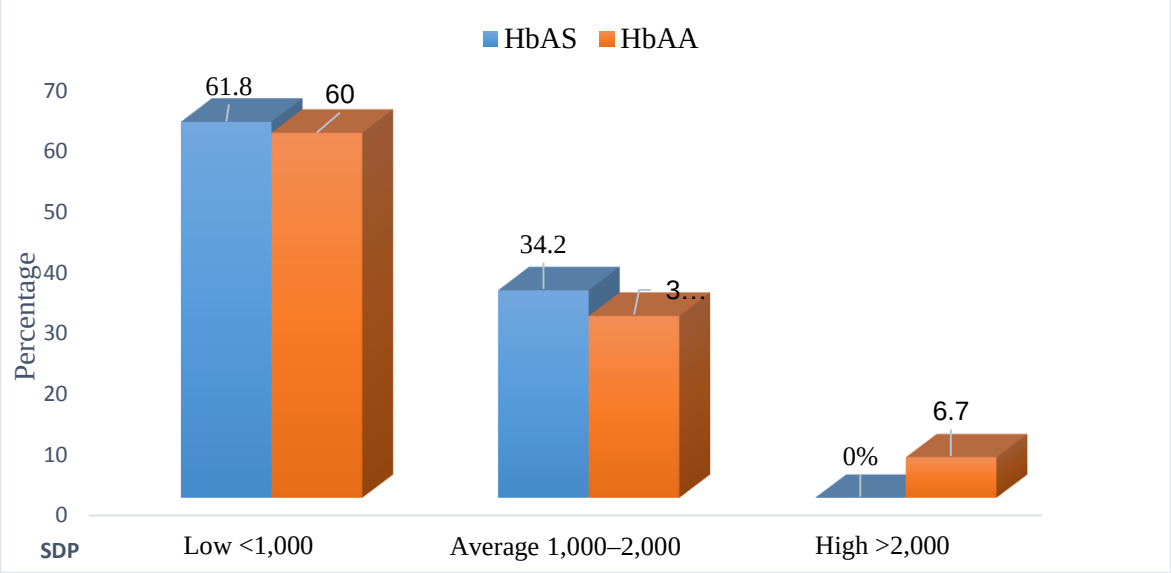


Figure 4-4 Monthly income of pregnant women with and without SCT in Sudanese pounds (SDP).

Table 4-3 Haematological parameters of the women with SCT (HbAS) and without (HbAA) at baseline

	HbAS (mean ± SD)	HbAA (mean ± SD)	p-value
Haemoglobin (g/dl)	11.8 ± 1.4	12.2 ± 1.2	NS
PCV (%)	34.4 ± 3.7	36.2 ± 3.3	0.020
MCV (fl)	80.2 ± 7.0	83.6 ± 5.7	0.011
MCH (pg)	27.1 ± 2.8	28.4 ± 2.6	0.015
TWBC (x 10^3)	7.2 ± 1.9	7.1 ± 2.7	NS
Neutrophils (%)	67.1 ± 10.8	66.5 ± 9.3	NS

Lymphocytes (%)	26.9 ± 7.9	27.6 ± 8.2	NS
PLTs (x 10^3)	266.9 ± 67.0	273.4 ± 78.6	NS

NS = not significant; SD = standard deviation

Table 4-4 Haematological parameters of the women with SCT (HbAS) and without (HbAA) at delivery.

	HbAS (mean±SD)	HbAA (mean±SD)	p-value
Haemoglobin (g/dl)	10.8 ± 0.6	11.6 ± 1.7	0.018
PCV (%)	31.2 ± 3.0	35.0 ± 5.1	0.000
MCV (fl)	93.6 ± 8.7	84.8 ± 7.1	0.000
MCH (pg)	26.0 ± 2.0	28.5 ± 2.9	0.000
TWBC (x 10^3)	8.2 ± 2.0	9.1 ± 3.2	NS
Neutrophils (%)	62.9 ± 8.3	68.0 ± 11.5	0.045
Lymphocytes (%)	27.2 ± 6.4	25.3 ± 9.9	NS
PLTs (x 10^3)	265.6 ± 42.7	192.6 ± 53.4	0.000

NS = not significant

Table 4-5 Haematological parameters of the babies of women with SCT (HbAS) and without (HbAA) at delivery.

	HbAS(babies)(mean±SD)	HbAA (babies) (mean±SD)	p-value
Haemoglobin (g/dl)	13.0 ± 4.4	14.9 ± 2.3	0.049
PCV (%)	36.1 ± 4.6	44.1 ± 6.8	0.000
MCV (fl)	100.0 ± 7.4	103.4 ± 7.7	NS

MCH (pg)	27.9 ± 1.9	34.8 ± 2.0	0.000
TWBC (x 10^3)	9.8 ± 1.6	10.5 ± 4.6	NS
Neutrophils (%)	62.4 ± 5.2	50.9 ± 13.7	0.004
Lymphocytes (%)	29.6 ± 5.5	41.8 ± 13.2	0.2
PLTs (x 10^3)	298 ± 37.1	225 ± 56.7	0.000

NS = not significant

Table 4-6 Birth weight, head circumference, special care baby unit (SCBU) admission and miscarriage rates among the babies born to the SCT (HbAS) and healthy (HbAA) groups.

	HbAS mean±SD	HbAA mean±SD	p-value
Birth weight (kg)	2.9 ± 0.2	3.2 ± 0.3	0.000
H. circumference (cm)	34.5 ± 0.7	34.1 ± 0.7	NS
Gestation (weeks)	38.04 ± 0.86	38.22 ± 0.59	0.179
	n (%)	**n (%)**	
SCBU admission	3 (13)	1 (2.5)	0.155
Miscarriage and stillbirth	3 (13)	1 (2.5)	0.836

SCBU = special care baby unit; NS = not significant

4.2.4 Analysis of results

Table 4.1 presents the demographic and clinical characteristics of the women with SCT (HbAS) and the women with normal haemoglobin (HbAA) at base line. There was no significant difference in mean age, weight, height or BMI at baseline between the two groups ($p>0.05$).

The women with SCT compared with the healthy control group had lower levels of educational achievements: more of the SCT group were illiterate and had achieved just primary education (44.1% vs. 25.0%; p=000), whereas fewer had attended middle and high school (47.1% vs. 55.0%; p>0.05) and university (8.8% vs. 20.0%; p=0.000) (Table 4.1 and Figure 4.1).

As with the first study of non-pregnant women, this study showed that consanguineous marriage was still common in Western Sudan, particularly for those with a genetic disorder and who were economically disadvantaged. Compared with the control group, there was a higher level of marriage to first and second-degree relatives among the women with SCT (64.7% vs. 43.3%; p<0.05) and their respective parents (61.8% vs. 56.7%; p<0.05) (Table 4.1 and Figure 4.2). The majority (78%) of the participants who had SCT had siblings with SCD.

The two groups had comparable employment and household income levels (p>0.05) (Table 4.2). The women with SCT were less likely to own their own house (41.2% vs. 60.0%; p>0.05) and were more likely to live with their relatives (38.2% vs. 25.0%; p>0.05) or in rented accommodation (20.6% vs. 15.0%; p>0.05) (Table 4.2 and Figure 4.4), although these data did not reach statistical significance.

Regarding haematological parameters, at enrolment, which was during the first trimester, the SCT group did not manifest SCA symptoms, and there were no differences in any of the haematological parameters between the SCT and control groups (Table 4.3).

At delivery (Table 4.4), the women with SCT compared with the control group had lower levels of haemoglobin (p=0.000), PCV (p=0.000), MCH (p=0.002) and neutrophil counts (p=0.045), and higher levels of MCV (p=0.000) and PLT counts (p=0.000). Similarly, at

delivery, the babies of the SCT women had lower haemoglobin levels (p=0.045), PCV (p=0.000) and MCH (p=0.000), and higher neutrophil (p=0.004) and PLT (p=0.000) counts (Table 4.5).

Birth outcome data are shown in Table 4.6. The mean birth weight of the babies born to the HbAS mothers was lower than that of the HbAA women's babies (p=0.000). There was no difference in mean head circumference between the two groups of babies (p>0.05). The levels of admission to the SCBU (13% vs. 2.5%; p>0.05) and the proportions of women who had miscarriages or stillbirths (13% vs. 2.5%; p>0.05) were higher in the SCT group than in the control group.

4.2.5 Discussion

This study investigated maternal and foetal outcomes in pregnancies that were complicated by SCT in Western Sudan. This region of the country is unique in that it has a high prevalence of SCD that overlaps with widespread poverty, malnutrition, illiteracy and consanguineous marriages. There is a lack of awareness about SCD and SCT before marriage. Most women do not ask about their potential husband's genetic status regarding SCD, nor do they ask about their potential in-laws' situation with regard to the disease. This is the case across the world in most areas where SCD is prevalent (Smith and Aguirre, 2012). Only recently in the West have screening programmes been established and premarital testing offered. In Sudan, there are no facilities for premarital testing or a screening programme at birth.

Consistent with previous findings (Daak *et al.,* 2016; Munsoor and Alabid, 2011), the majority of women in this study, with or without SCT, were married to their first or second cousins. However, the number was considerably higher (64.7% vs. 43.3%) in the SCT group than in the control group (Figure 4.2). These findings were similar to those

reported in a study performed in Oman, where it was found that 24.1% of marriages were between first cousins, and 20.4% were within a tribe (Rajab and Patton, 2000).

The current study found that many of the women with SCT had poor educational backgrounds (illiterate or primary school level) and that women with chronic disease, low education levels, and low socioeconomic backgrounds, often face difficulty to find a partner from within their family circles (Figure 4.1).

The HbAS and HbAA pregnant women in our cohort had equal employment opportunities and comparable earnings. However, most of the HbAS women did not have a university education or own their own houses. This may have been because families affected by SCD were not able to send their children to a university because of the financial burden associated with higher education.

Although the number of women with SCT in our cohort was small, the findings were consistent with previous observations, which indicated that individuals with HbAS suffered from employment, health insurance and marriage discrimination (Kose and Kose, 2019). However, another study that compared people with SCTs and healthy controls did not find a difference in socioeconomic status (Adeyemi *et al.,* 2009).

The current study results showed that siblings of the HbAS group were more likely to be affected by HbS than the siblings of their HbAA counterparts (65% vs. 13%) (Figure 4.3). Munsoor and Alabid reported similar findings in the same region. In this region of Sudan, it appears that the sickle cell gene is propagated through the tribal habit of marriage between relatives. Consanguineous marriage supports the spread of the disease. Tailored health education and public awareness programmes should address this issue. There is a charity group that focuses on this programme and which seems to be successful, and this may lead to improvement. As a result of the charity's efforts, attitudes

and knowledge are changing, and affected people are positively disposed toward premarital testing and screening programmes, as is applied in Bahrain (Al Arrayed and Al Hajeri, 2012).

While collecting information, we conducted focus-group discussions in the area of study. The leader of the well-known tribe in the region said that he had three wives. Two of them were his cousins. The third was unrelated to him. The disease had affected most of the children of the two wives who were his cousins. Apparently, all of his children from his third wife were healthy, although they may be carriers. He has agreed to participate in the awareness programme we will conduct in 2023 in the region.

At baseline, which was during the women's first trimester, the two groups of women had comparable weight, height, BMI and blood glucose levels. Perhaps this was to be expected, as the two groups had similar meal frequencies (most of them ate breakfast, lunch and/or dinner regularly) and consumed analogous types and amounts of foods (meat, eggs, bread, rice, fruits and vegetables). Moreover, because they belonged to closely related tribes and had the same religious affiliations, they held the same food beliefs and taboos.

The two groups of women had similar levels of haemoglobin at baseline (first trimester). However, the SCT group had significantly lower percentages of PCV, MCV and MCH, which indicated marginal iron deficiency anaemia (IDA).

It is recommended that levels of haemoglobin in pregnant women should range between 11.5 g/dl to 13g/dl, but a haemoglobin level of 10g/dl to 12 g/dl during the third trimesters are regarded as common reference range due to haemodilution in pregnancy. Not like what was considered earlier that pregnant women with a haemoglobin level of

less than 11g/dl during the third trimesters are regarded as anaemic (Kadry et al., 2018; WHO/UNICEF/United Nations University (UNU), 1998, 2001).

It was not surprising that the women who participated in this study were either iron deficient or anaemic, because the western region of Sudan is semi-arid, and hunger and malnutrition are widespread. In addition, Sudan has the highest rate of pregnancy anaemia (53%) in Africa (Adam *et al.*, 2018).

Consistent with previous reports (Hamdi *et al.*, 2002; Manzar, 2000; Tsaras *et al.*, 2009), this study found that at delivery, the women with SCT had significantly lower PCV percentages and haemoglobin and MCH concentrations than did their healthy counterparts (HbAA), which indicated that the women with SCT were anaemic at delivery.

In accordance with a recommendation of the WHO (2011), iron and iron-folate tablets are available free of charge to pregnant women in Sudan (Abdullahi *et al.*, 2014). Therefore, it was thought that the women in this study were taking these supplements, so the low haemoglobin level was not anticipated. It is possible that the women were not provided with the necessary instructions on the use of the supplements or they failed to adhere strictly to the given guidance and advice. Also, they might have stopped taking the supplements because of the side effects.

There are reports that compliance with the dosage instructions for iron/folic acid supplements is positively related to maternal education level, appropriate antenatal education and care, knowledge about IDA and the use of these supplements, and regular antenatal care visits (Abdullahi *et al.*, 2014; Gebremariam *et al.*, 2017; Solomon *et al.*, 2021; Tarekegn *et al.*, 2019).

Also, there is evidence that intermittent supplementation with iron or iron/folic acid prevents IDA, while compliance is enhanced because the side effects associated with the taking of iron are reduced (Peña-Rosas *et al.*, 2014).

IDA in SCD has been reported in India and Jamaica (Mohanty *et al.,* 2008; Serjeant, 2001). Mohanty *et al.* (2008) reported that 25% of women with SCT in India were iron deficient. These researchers reported that IDA should be confirmed either by direct measurement of the ferritin level in blood or the measurement of the zinc protoporphyrin:heme ratio and haemoglobin levels (Mohanty *et al.,* 2008). They found that iron supplementation at doses of 3mg/kg showed good improvement.

It is known that low levels of MCV indicate iron deficiency. However, in this study, it was found that MCV levels in the SCT group were high. Similar findings were reported by Mohanty *et al.* (2008) among Indian women, among Jamaican HbSS patients (Serjeant, 2001) and among patients in El Fashir hospital in Western Sudan (Adam *et al.,* 2019).

The values for the haematological parameters of the babies who were born to the women with SCT and the healthy controls closely mirrored those of their mothers at delivery. The haemoglobin, PCV and MCH levels of the former were significantly lower than those of the latter group. However, in both groups, in agreement with earlier studies (Dapper and Didia, 2006; Nneli *et al.,* 2011), the values of the above-mentioned three haematological parameters were higher in the babies than in the mothers.

Maternal and neonate blood-iron biomarkers do not seem to correlate well (Sanni *et al.,* 2020), and the haematological reference values of cord blood/neonates vary considerably (Alkindi *et al.,* 2011; Angelo *et al.,* 2021; Nneli *et al.,* 2011). Therefore, it is not possible to state with confidence whether or not the babies born to the mothers who participated

in the study were anaemic or iron deficient. Maternal SCT had adverse effects on the haematological parameters of these babies. The haemoglobin, PCV, MCV and MCH values of the babies of mothers with SCT were significantly lower than those of the neonates of the healthy control group and the normal cord blood reference ranges that have been reported from Sudan (Elgari and Waggiallah, 2014).

The causes of admission to SCBU were:

In three babies for HbAS mothers, one was due to neonatal physiological jaundice that occurred the second day after birth which required double phototherapy for 2 days. The second baby had APGAR of 6 at one minute and 7 at 5 minutes he was lethargic so he was kept for 24 hours in SCBU after proper resuscitation and management. His weight was 2.5 kg. He was discharged to the postnatal ward. The third baby had low blood glucose at birth 1.5 mmol and was persistently low after dextrose infusion, he was not a baby of a gestational diabetic mother but his weight was 3.5 kg. He was kept for 5 days in SCBU and discharged to the post-natal ward where he was breastfed by his mother and was well thereafter.

1 baby for HbAA mother had physiological neonatal jaundice required single phototherapy for 24 hours and was discharged to the postnatal ward.

Incidences of miscarriage and stillbirth, admissions to the SCBU and low birth weights were higher in the SCT group than in the normal group. These findings were consistent with those of previous studies, which reported anaemia and neonatal/foetal mortality (Hamdi *et al.,* 2002; Taylor *et al.,* 2006), low birth weight (Hoff *et al.,* 1983; Taylor *et al.,* 2006), prematurity and pre-eclampsia (Wilson *et al.,* 2020), intrauterine foetal hypoxia (Manzar, 2000) and placental infarction and calcification (Taylor *et al.,* 2006)

in pregnancies complicated by SCT. A retrospective study that was performed for the period 2000 to 2005 of SCT deliveries at St Thomas' Hospital in London reported that 16.8% (79/471) of the babies were small for their gestational age (Tan *et al.,* 2008).

It is not clear why SCT pregnancy is associated with adverse birth outcomes. Nevertheless, several studies have highlighted that iron deficiency and anaemia are risk factors for adverse pregnancy outcomes and preterm delivery, prematurity and low birth weight (Kadry *et al.,* 2018; Kidanto *et al.,* 2009; Sukrat *et al.,* 2013), while placental pathological changes – infarction and calcification – may also play a role (Jung *et al.,* 2018; Taylor *et al.,* 2006).

Due to logistical reasons and financial constraints, during this study, reticulocyte count was not determined, and the placentae were not evaluated for pathological changes (placental infarction and calcification). Additionally, there was no postpartum follow-up. These limitations will be addressed in our forthcoming study.

4.2.6 Conclusions

This study has shown that SCT in pregnancy is associated with adverse maternal and foetal/neonatal outcomes. Nonetheless, it is unclear whether the risk is restricted to undernourished women with the condition in Western Sudan or whether it is universal to all pregnant women with SCT. At present, most obstetricians in Sudan believe that SCT is a benign condition and that women with it can enjoy normal pregnancies. Consequently, these women are not given specialist antenatal or postnatal care. This perception must be changed.

Women with SCT who embark on pregnancy should be regarded as a high risk group and given preconception advice and multidisciplinary antenatal and postnatal care. Screening programmes should be implemented in the prenatal clinic so that pregnant

women are diagnosed early in their pregnancy and classified as high risk if they carry the sickle cell gene. The haemoglobin electrophoresis machine offers a better diagnosis method than a simple solubility test. A repeat test can be performed during the first trimester to confirm the results. Newly diagnosed patients should be counselled, their spouse should be tested, and a postnatal test should be offered for the baby at birth and at six months.

Chapter 5

5. Blood fatty acid levels among Sudanese pregnant women with and without SCT

5.1. Introduction

5.1.1 Fatty acids

Fatty acids are hydrocarbon chain compounds that have a methyl group (CH_3) at one end and a carboxylic group (COOH) at the other (Sprecher, 2000). Most of the fatty acids that are found in human tissues contain double bonds (unsaturated) or do not (saturated). The number of double bonds can be one (monounsaturated) or two or more (polyunsaturated).

The International Union of Pure and Applied Chemistry (IUPAC) classifies unsaturated fatty acids into three families (n3/ω3, n6/ω6, and n9/ω9) based on the position of the first double bond and counting from the methyl end of the hydrocarbon chain (Ratnayake and Galli, 2009).

5.1.1.1 Saturated fatty acids

Saturated fatty acids (SFA) are often classified as short (fewer than six carbon atoms), medium (six to 12 carbon atoms), or long-chain (14 or more carbon atoms). Human milk contains significant amounts of medium-chain (capric, C10:0, and lauric, C12:0) fatty acids, particularly in women whose day-to-day diet is dominated by carbohydrates. In contrast, short- and medium-chain fatty acids are hardly found in human tissue. However, trace levels of lauric (C12:0) and myristic (C14:0) are detected in individuals and population groups who consume a lot of carbohydrates (Ratnayake, 2009).

Circulating (plasma) and cell membrane (blood cells and other tissues) lipids contain significant amounts of palmitic (C16:0) and stearic (C18:0) and trace levels of arachidic (C20:0), behenic

(C22:0) and lignoceric (C24:0) fatty acids. The latter three fatty acids are found in appreciable amounts in sphingolipids.

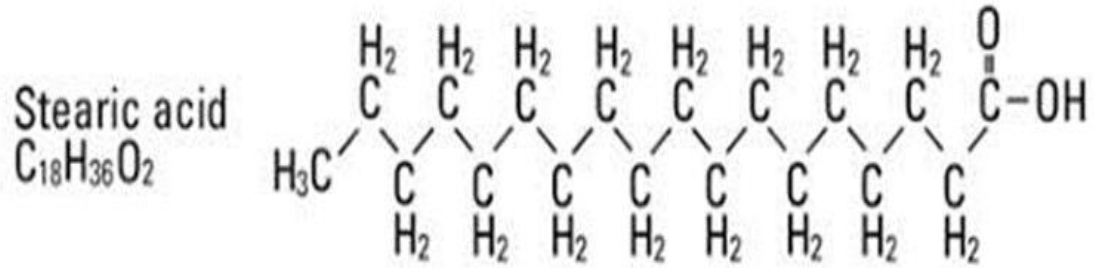


Figure 0-1 A schematic example of a saturated fatty acid (stearic acid, 18:0).

5.1.1.2 Monounsaturated fatty acids

Monounsaturated fatty acids are long-chain (C16 or longer) carboxylic acids with one double bond. The hydrogen atoms attached to the double bond are in the cis configuration, which means that they are located on the same side of the double bond. The monounsaturated fatty acids palmitoleic (C16:1n7), cis-vaccenic (C18:1n7), and oleic (C18:1n9) are found in considerable amounts in human tissue, and neutral milk lipids and glycerophospholipids. In contrast, sphingolipids contain significant nervonic (C24:1n9) and trace gondoic (C20:1n9) and erucic (C22:1n9) acid levels. Mammals, including humans, can synthesise long-chain monounsaturated fatty acids from carbohydrates. The primary dietary sources of monounsaturated fatty acids are olives, peanuts, sunflowers, safflowers, nuts, avocadoes and their oils, and canola oil (Siscovick *et al.*, 2017).

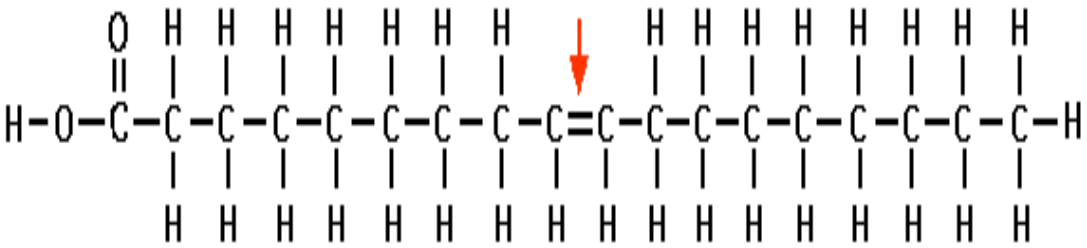


Figure 0-2 A schematic example of a monounsaturated fatty acid (oleic; adapted from Mukumbo & Muchenje, 2016).

5.1.1.3 Polyunsaturated fatty acids

Polyunsaturated fatty acids (PUFAs) are long-chain carboxylic acids with two or more double bonds. The PUFAs found in humans are mainly the n7, n9, n6, and n3 fatty acid families. The n9 PUFAs, mead (C20:3n9) and di-homo mead (C22:3n9) acids, are synthesised by mammals from carbohydrates or the precursor fatty acids, C16:1n7 and C18:1n9, through elongation (addition of two carbon atoms) and desaturation (insertion of double bonds). In contrast, mammals cannot synthesise the parent compounds of the n6 (LA, C18:2n6) and n3 (alpha-linolenic, C18:3n3) families de novo because they do not have the required enzymes, delta12 and delta15 (see Figure 5.3). Consequently, these essential fatty acids must be obtained from foods. The primary sources of alpha-linolenic acid (C18:3n3) are linseed, rapeseed, pumpkinseed, walnut and soybean oils, and green vegetables. LA (C18:2n6) is obtained from vegetable oils (sunflower, safflower, corn, soybean), nuts, seeds, eggs, and meat (Crawford *et al.*, 1993).

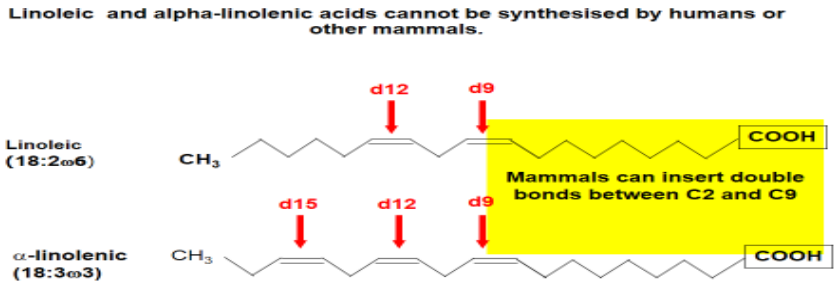


Figure 0-3 Schematic diagrams of the parent compounds of the n6 (LA, C18:2n6) and n3 (alpha-linolenic, C18:3n3) families (adapted from Professor Kebreab Ghebremeskel).

Mammals, including humans, can synthesise the long-chain n6 and n3 PUFAs from their respective parent compounds through sequential elongation (addition of two carbon atoms) and desaturation (insertion of double bonds) (Figure 5.4).

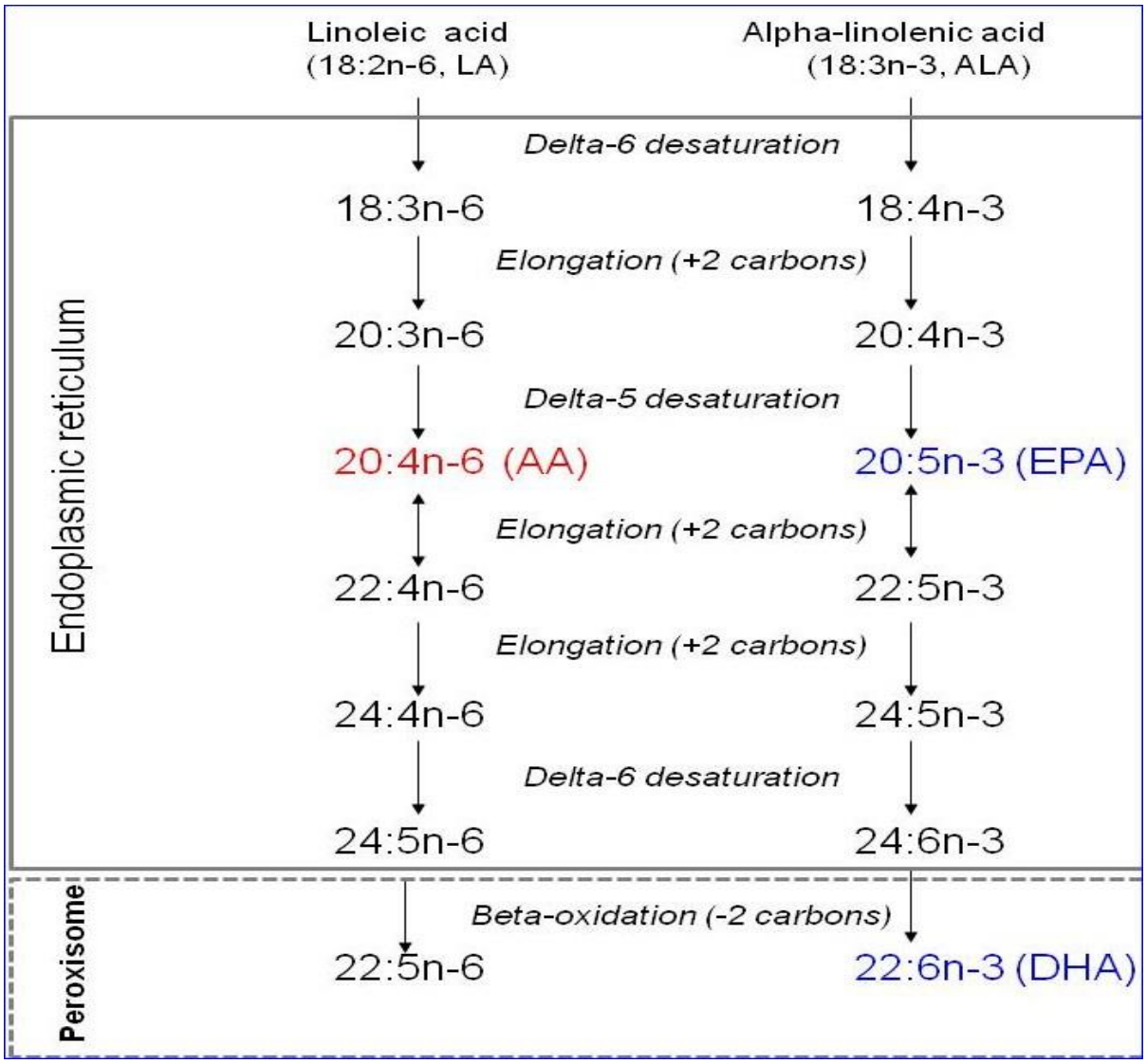


Figure 0-4 Elongation and desaturation of LA and alpha-linolenic acid to their respective long-chain metabolites. ((Adapted from Professor Kebreab Ghebremeskel).

The long-chain n6 and n3 PUFAs are vital components of the cellular and sub-cellular membrane lipids. AA (C20:4n6) comprises 15% to 20% of the total fatty acids of the polar

phospholipids found in all biological membranes. In contrast, DHA (C22:6n3) is found in appreciable amounts in the brain, testes, sperm and retina (Tinoco, 1982). The levels of DHA in the outer photoreceptor segment (Fleischer and Anderson, 1983) and the cerebral grey matter (O'Brian and Sampson, 1965) account for about 60% and 30% of the body's DHA, respectively. Similarly, synaptosomes, synaptic vesicles, mitochondria and microsomes contain high levels of DHA (Suzuki, Manabe and Wada, 1997). High levels of saturated and monounsaturated fatty acids, and low levels of AA and DHA, are found in the brain's white matter and myelin fractions (Fleischer and Rouser, 1965). The long-chain n3 and n6 PUFAs, AA, DHA, dihomo-gamma-linolenic, EPA (C20:5n3) and docosapentaenoic (DPA, C22:5n3) acids are precursors of hormone-like bioactive lipids (Figure 5.5). These lipids are known as eicosanoids and they play critical roles in immunity and inflammatory responses, gene expression, vascular tone, oxidative stress and antioxidant defence (Ghebremeskel *et al.*, 2006).

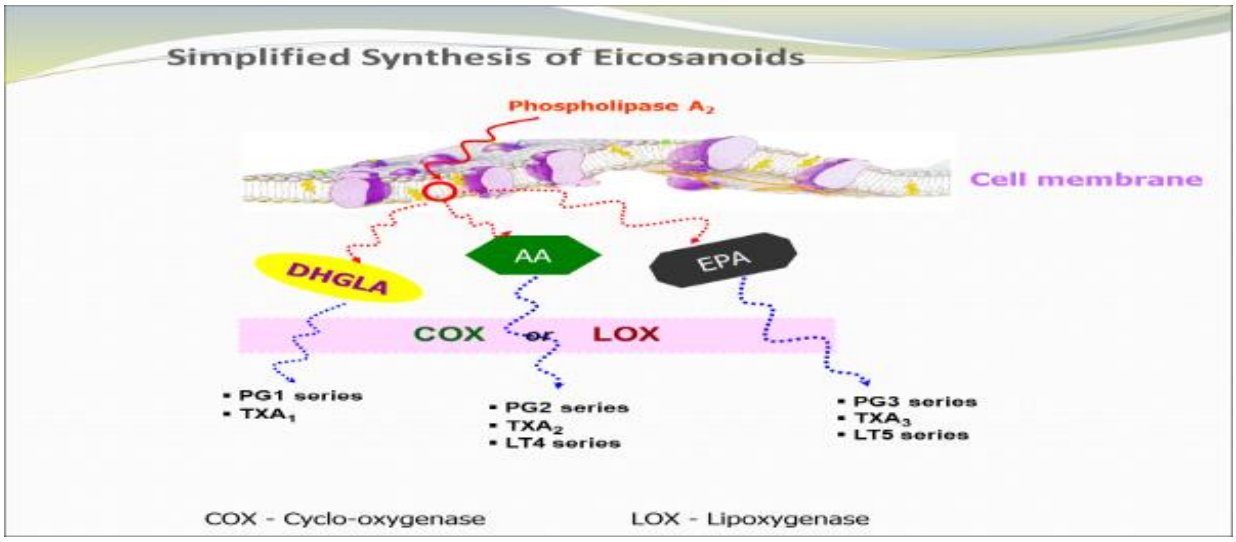


Figure 0-5 Simplified representation of the synthesis of some eicosanoids ((adapted from Professor Kebreab Ghebremeskel).

5.1.2 Fatty acids and SCD

Several studies have reported that some of the clinical manifestations of SCD, such as excessive blood cell adhesion and blood cell aggregation, are associated with a perturbation of membrane lipids or fatty acids. Heightened exposure of phosphatidylserine in the outer leaflet of the lipid bilayer of the plasma membrane and associated loss of membrane phospholipid asymmetry has been reported (Barber *et al.,* 2009; Kuypers, 2007; Setty *et al.,* 2000). Steady-state Nigerian and British patients with SCA (HbSS) have been reported to have abnormal levels of long-chain n3 and n6 PUFAs in RBCs and plasma (Ren *et al.,* 2005a) and PLTs and mononuclear cells (Ren *et al.,* 2005b).

A similar abnormality has been observed in patients with sickle cell HbC (Ren *et al.,* 2005c). Indeed, dietary supplementation with the DHA n3 fatty acid has been shown to ameliorate the fatty acid abnormality and to have several benefits: Daak *et al.* (2013) reported that it reduced the occurrence of severe anaemia and clinical vaso-occlusive events, and the number of required blood transfusions; Hosni Abdel Hamed (2018) found that such supplementation reduced the number of vaso-occlusive crises and required blood transfusions, and cut school absenteeism; and Okpala *et al.* (2011) reported that its use reduced the number of crises and steady-state haemolyses that occurred.

5.1.3 Pregnancy and n3 and n6 fatty acids

A pregnant woman's body demands increased levels of long-chain n3 and n6 PUFAs, particularly AA and DHA, to grow and develop pregnancy-associated maternal organs breasts and pelvic organs) and the vital foetal organs, including the central nervous system (brain, retina, etc.). The placenta or foetus cannot synthesise AA or DHA, and hence they are obtained from maternal circulation. This maternal-to-foetal transfer often leads to a significant reduction

in maternal blood levels of AA and DHA in healthy pregnant women (Holman *et al.,* 1991; Hornstra *et al.,* 1995), particularly in those who do not consume foods rich in the aforementioned fatty acids. In contrast, pregnant women from populations with a high intake of long-chain n3 fatty acids show longer gestational ages and have fewer preterm deliveries and less pregnancy-induced hypertension than those whose intake of these fatty acids is low (Olsen *et al.,* 1986).

The placental supply of AA and DHA to the foetus may be reduced if the mother has pre-eclampsia (Qiu *et al.,* 2007), chronic diseases such as diabetes (Ghebremeskel *et al.,* 1997, 2004; Min *et al.,* 2006), fatty acid metabolism disorder or placental infarction. Babies born before the term date and at low birth weight have reduced levels of AA and DHA (Crawford *et al.,* 1976; Koletzko *et al.,* 1991; Leaf *et al.,* 1991). A study conducted by our research group revealed a direct relationship between levels of AA and birth weight, and levels of DHA and head circumference (Leaf *et al.,* 1992a, and 1992b).

There are no published high-quality data on the fatty acid status of Sudanese pregnant women with chronic diseases such as SCD, diabetes or hypertension, or for those without chronic diseases. Hence, there was a need for the current study.

5.2 Aim of this study

The aim was to investigate whether or not pregnancy complicated by the presence of SCT (HbAS) was associated with abnormal fatty acid status. Blood fatty acid levels were used as markers of status.

5.3 Subjects and methods

5.3.1 Subjects

The women who had been enrolled for the haematology study (Chapter 4) were asked to take part also in the fatty acid study. The recruitment procedure for that study is outlined in Chapter 4, section 4.2.2.1. That study enrolled 34 women with SCT (HbAS) and 60 healthy (HbAA) women for comparison. Of the 34 women with SCT who were enrolled for that study and therefore had agreed to give blood samples, 17 refused to give second samples for fatty acid analysis. Similarly, of the 60 healthy women who had agreed to take part, 20 withdrew their consent and 20 refused to give second samples for fatty acid analysis. Consequently, baseline blood samples (5ml) were taken from 17 women with SCT and 20 healthy controls for fatty acid analysis. At delivery, blood samples (5ml) were taken for fatty acid analysis from 15 SCT and 15 healthy women. The two SCT women who did not give blood at delivery had suffered miscarriages, as had one of the healthy controls. The other twenty four healthy women who did not give blood at delivery declined to do so. The flow chart in Figure 5.6 explains the recruitment at each stage.

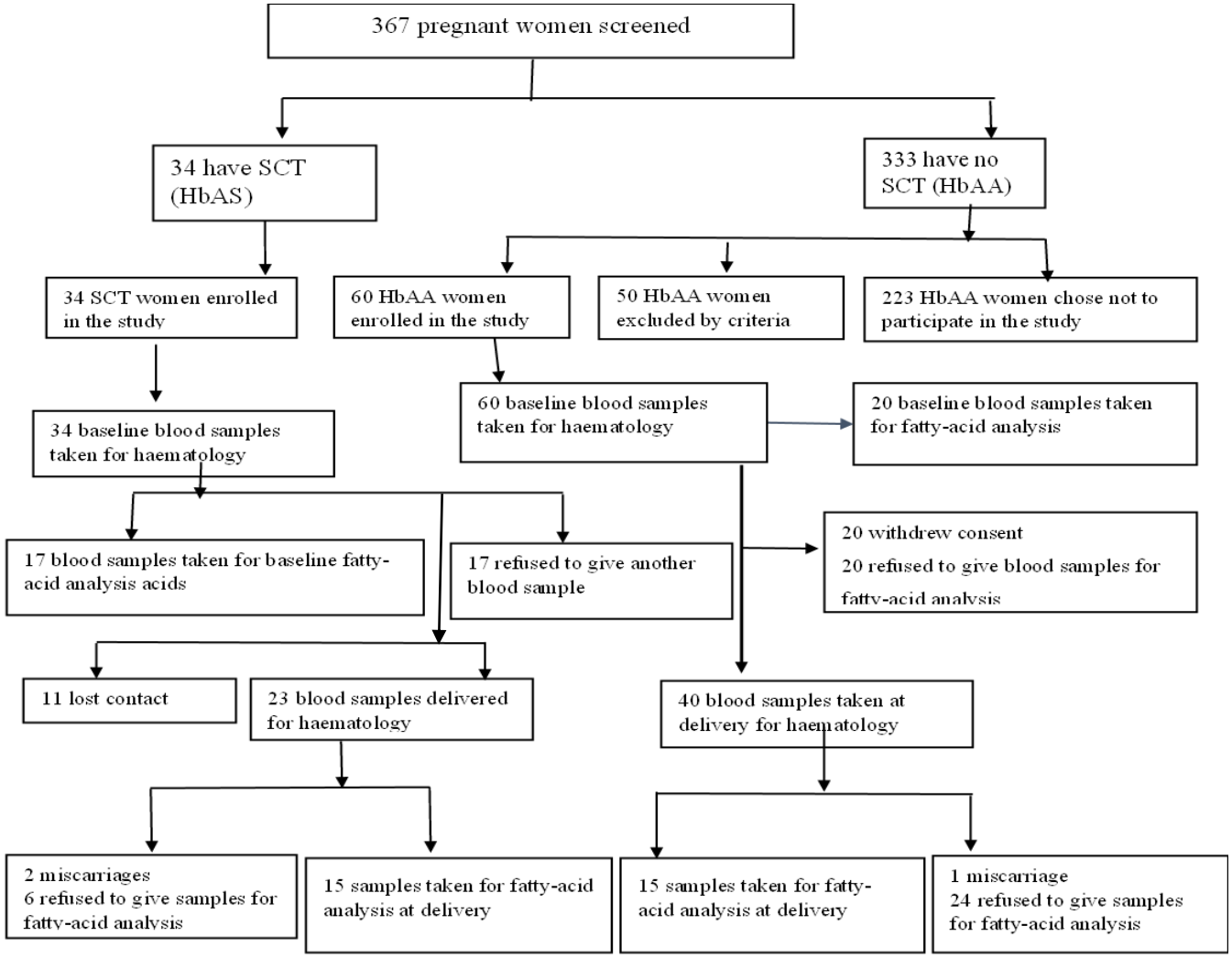


Figure 0-6 Flow chart shows the recruitment of participants for the haematology study explained in Chapter 4 and for the fatty acid analysis explained in this chapter.

5.3.2 Methods

5.3.2.1 Fatty acid analysis

Total lipids were extracted from 500µl of plasma (baseline samples) and the same volume of RBCs (delivery samples) and analysed for their fatty acid content. The methods that were used to extract the lipids, prepare FAMEs and separate and quantify the FAMEs are described in Chapter 2.

5.4 Data analysis

The fatty acid data were checked for normal distribution and subsequently expressed as mean ± SD. The mean values of the two groups were tested for statistical significance through the application of unpaired t-tests (independent t-tests), and $p<0.05$ were considered significant. The data were analysed using the IBM SPSS Statistics program, version 26 (IBM SPSS Ltd, Woking, Surrey, UK).

5.5 Results

5.5.1 Baseline fatty acids

Plasma fatty acid data for the women with SCT (HbAS) and without (HbAA) are presented in Table 5.1. Compared with their healthy counterparts, the women with SCT had higher levels of palmitic ($p=0.016$), stearic ($p=0.018$) and total SFA ($p=0.002$). Of the monounsaturated fatty acids, the amount of oleic acid was higher ($p=0.05$) and Vaccenic acid lower ($p=0.025$) in the SCT group compared with the healthy group. The amounts of n6 metabolites ($p=0.04$), total n6 fatty acids ($p=0.009$), and the particular n6 fatty acids, gamma-linolenic ($p=0.004$) and adrenic ($p=0.045$), were higher in the SCT group than in the healthy group. Similarly, the women with

SCT compared with their healthy counterparts had higher levels of EPA (p=0.016) and DPA (p=0.016). The two groups of women had comparable levels of AA (C20:4n6) and DHA (C22:6n3).

Table 0-1 Baseline levels of plasma fatty acids in pregnant Sudanese women with SCT (HbAS) and without (HbAA)

Fatty acids	HbAS (n=17)	HbAA (n=20)	p-value
14:0 (myristic)	0.77 ± 0.35	0.73 ± 0.23	0.310
16:0 (palmitic)	25.5 ± 1.8	24.4 ± 1.3	0.016
18:0 (stearic)	6.6 ± 0.8	6.1 ± 0.7	0.018
22:0 (behenic)	0.48 ± 0.18	0.47 ± 0.27	0.465
24:0 (lignoceric)	0.22 ± 0.08	0.26 ± 0.12	0.097
Total saturated fatty acids	33.6 ± 1.5	32.0 ± 1.6	0.002
16:1n7 (palmitoleic)	1.4 ± 0.6	1.4 ± 0.7	0.429
18:1n9 (oleic)	19.8 ± 2.4	18.6 ± 2.0	0.050
18:1n7 (vaccenic)	1.3 ± 0.4	2.0 ± 1.3	0.025
24:1n9 (nervonic)	0.15 ± 0.06	0.12 ± 0.08	0.083
Total monounsaturated fatty acids	22.7 ± 2.9	22.1 ± 1.8	0.336
18:2n6 (LA)	27.4 ± 2.5	26.1 ± 2.4	0.062
18:3n6 (gamma-linolenic)	0.59 ± 0.25	0.40 ± 0.14	0.004
20:3n6 (dihomo-gamma-linolenic)	1.5 ± 0.4	1.4 ± 0.2	0.263
20:4n6 (AA)	8.3 ± 1.5	7.5 ± 1.5	0.056

22:4n6 (adrenic)	0.31 ± 0.12	0.22 ± 0.18	0.045
22:5n6 (osbond)	0.74 ± 0.29	0.74 ± 0.32	0.475
N6 metabolites	11.4 ± 1.5	10.5 ± 1.5	0.048
Total n6	38.8 ± 3.0	36.4 ± 2.8	0.009
18:3n3 (alpha-linolenic)	0.11 ± 0.05	0.08 ± 0.08	0.121
20:5n3 (EPA)	0.03 ± 0.02	0.02 ± 0.02	0.016
22:5n3 (DPA)	0.08 ± 0.03	0.05 ± 0.05	0.016
22:6n3 (DHA)	0.99 ± 0.34	0.99 ± 0.39	0.497
Total n3	1.06 ± 0.24	1.10 ± 0.31	0.329
N3 metabolites	1.1 ± 0.24	1.1 ± 0.31	0.329
N6/N3	32.4 ± 9.7	30.1 ± 9.3	0.486

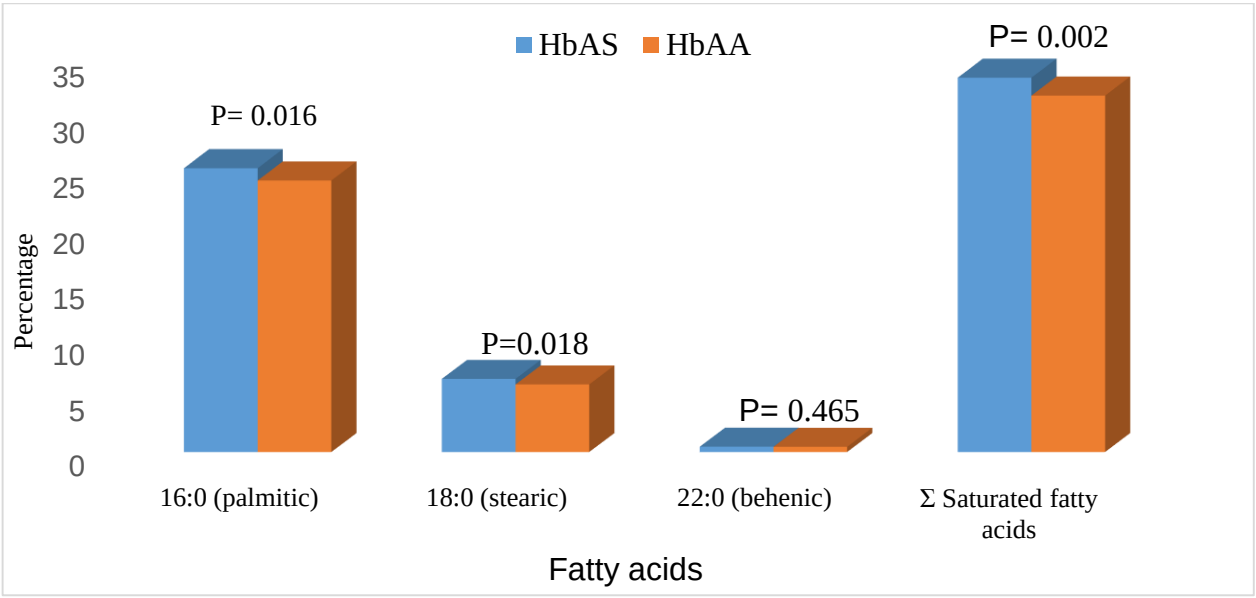


Figure 0-7 Baseline levels of saturated fatty acids in the plasma of HbAS and HbAA groups;

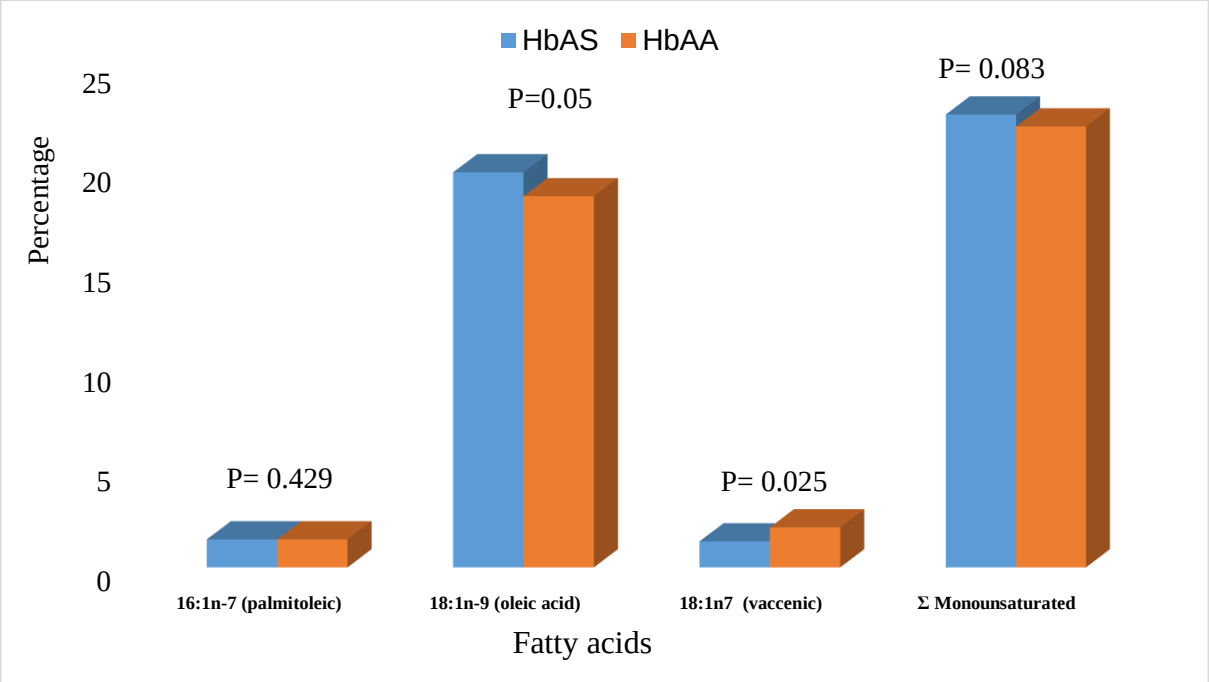


Figure 0-8 Baseline levels of monounsaturated fatty acids in the plasma of HbAS and HbAA groups.

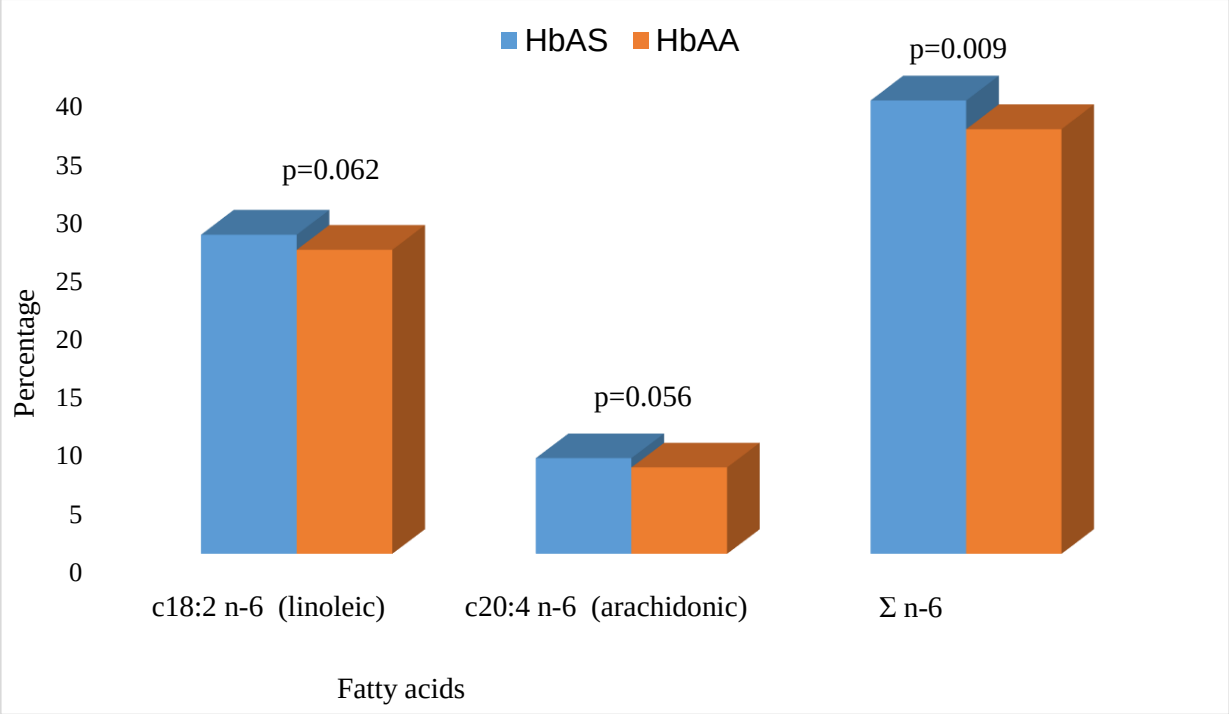


Figure 0-9 Baseline levels of n6 fatty acids in plasma of HbAS and HbAA groups.

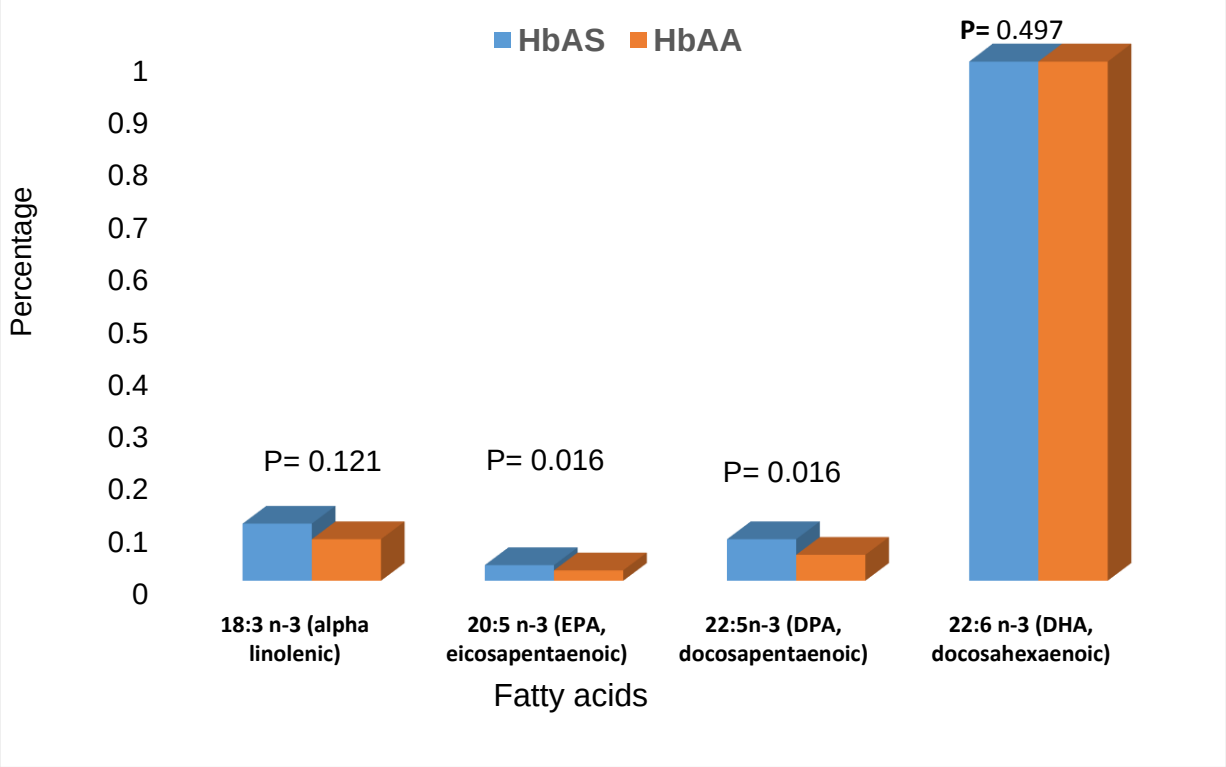


Figure 0-10 Baseline levels of n3 fatty acids in plasma of HbAS and HbAA groups.

5.5.2 Fatty acids levels at Delivery

Fatty acid levels in the RBCs of the women with SCT (HbAS) and without (HbAA) are shown in Table 5.2. The women with SCT compared with the healthy control group had higher levels of stearic (p=0.000), behenic (p=<0.001), lignoceric (p=0.011) and total saturated (p=0.001) fatty acids. Similarly, levels of total monounsaturated (p<0.001), oleic (p<0.001), vaccenic (p=0.005) and nervonic (p<0.001) fatty acids were higher in the women with SCT, as were HbAS, total n6 (p=0.004) and gamma-linolenic (p=0.010) acids, LA (p=0.008), AA (p=0.043) and n6 metabolites (p=0.016). In addition, the SCT group had higher levels of EPA (p=0.025) and α-linolenic (p=0.034) acids.

Table 0-2 Percentage levels of fatty acids in RBCs of pregnant Sudanese women with SCT and without at delivery of their babies

Fatty acids	HbAS (n=15) (mean±SD)	HbAA (n=15) (mean±SD)	p-value
14:0 (myristic)	0.48 ± 0.23	0.42 ± 0.15	0.419
16:0 (palmitic)	29.7 ± 3.1	28.8 ± 3.9	0.547
18:0 (stearic)	16.2 ± 3.2	10.4 ± 1.4	0.000
22:0 (behenic)	1.4 ± 0.2	1.1 ± 0.2	<0.001
24:0 (lignoceric)	4.4 ± 1.4	3.0 ± 1.0	0.011
Total saturated fatty acids	52.4 ± 4.8	43.8 ± 3.5	<0.001
16:1n7 (palmitoleic)	0.30 ± 0.19	0.38 ± 0.33	0.510
18:1n9 (oleic)	13.3 ± 3.0	9.1 ± 2.7	<0.001
18:1n7 (vaccenic)	1.1 ± 0.4	0.72 ± 0.22	0.005

24:1n9 (nervonic)	1.6 ± 0.3	1.1 ± 0.3	<0.001
Total monounsaturated	16.4 ± 3.6	11.4 ± 3.2	<0.001
18:2n6 (LA)	6.3 ± 2.0	4.3 ± 1.2	0.008
18:3n6 (gamma-linolenic)	0.61 ± 0.23	0.41 ± 0.12	0.010
20:3n6 (dihomo-gamma-linolenic)	0.50 ± 0.23	0.39 ± 0.16	0.164
20:4n6 (AA)	2.9 ± 1.0	2.1 ± 0.84	0.043
22:4n6 (adrenic)	0.61 ± 0.30	0.42 ± 0.19	0.067
22:5n6 (osbond)	0.27 ± 0.12	0.20 ± 0.13	0.172
n6 metabolites	5.0 ± 1.4	3.6 ± 1.2	0.016
Total n6	11.2 ± 2.9	7.9 ± 2.2	0.004
18:3n3 (α-linolenic)	0.01 ± 0.00	0.02 ± 0.00	0.034
20:5n3 (EPA)	0.03 ± 0.01	0.02 ± 0.01	0.025
22:5n3 (DPA)	0.04 ± 0.02	0.07 ± 0.05	0.071
22:6n3 (DHA)	0.23 ± 0.12	0.15 ± 0.10	0.082
Total n3	0.47 ± 0.51	0.39 ± 0.22	0.300
n3 metabolites	0.33 ± 0.14	0.27 ± 0.11	0.224
n6/n3	35.2 ± 10.4	30.5 ± 11.3	0.286

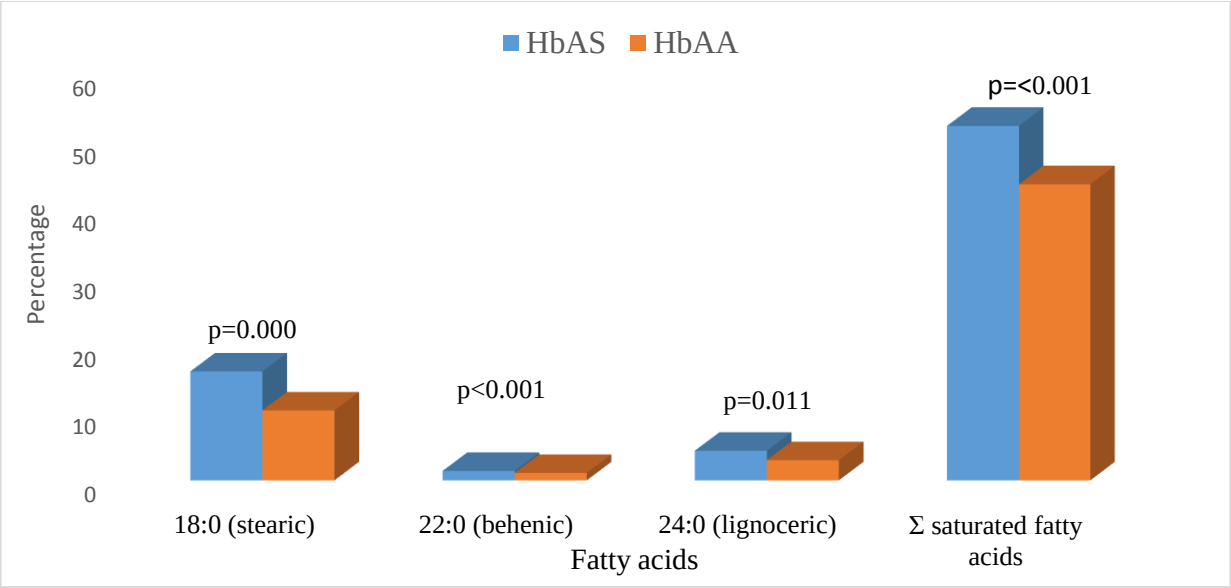


Figure 0-11 Levels of saturated fatty acids in RBCs of HbAS and HbAA women at delivery.

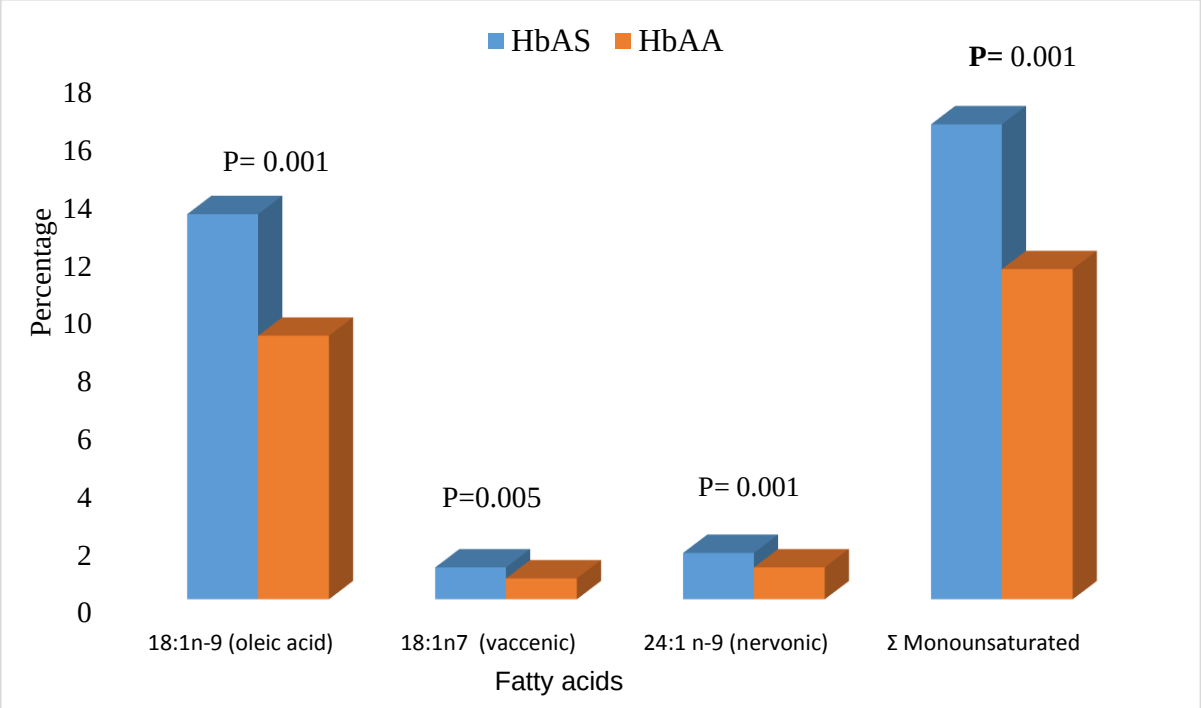


Figure 0-12 Levels of monounsaturated fatty acids in RBCs of HbAS and HbAA women at delivery.

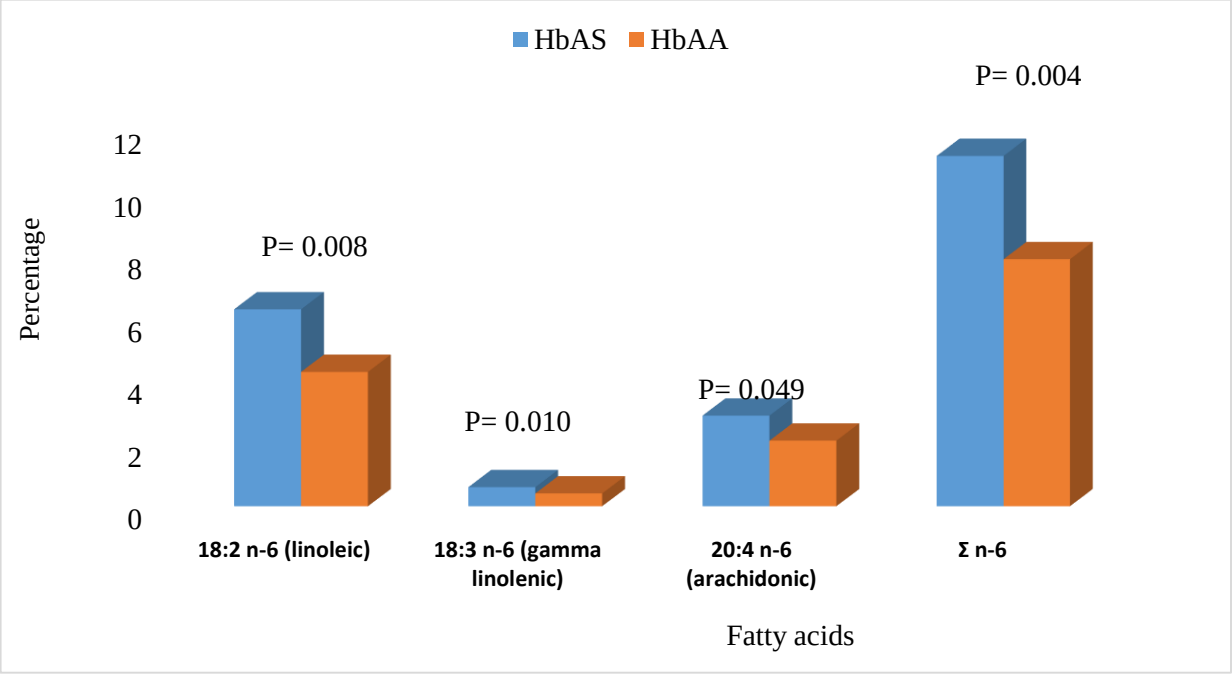


Figure 0-13 Levels of n6 fatty acids in RBCs of HbAS and HbAA women at delivery.

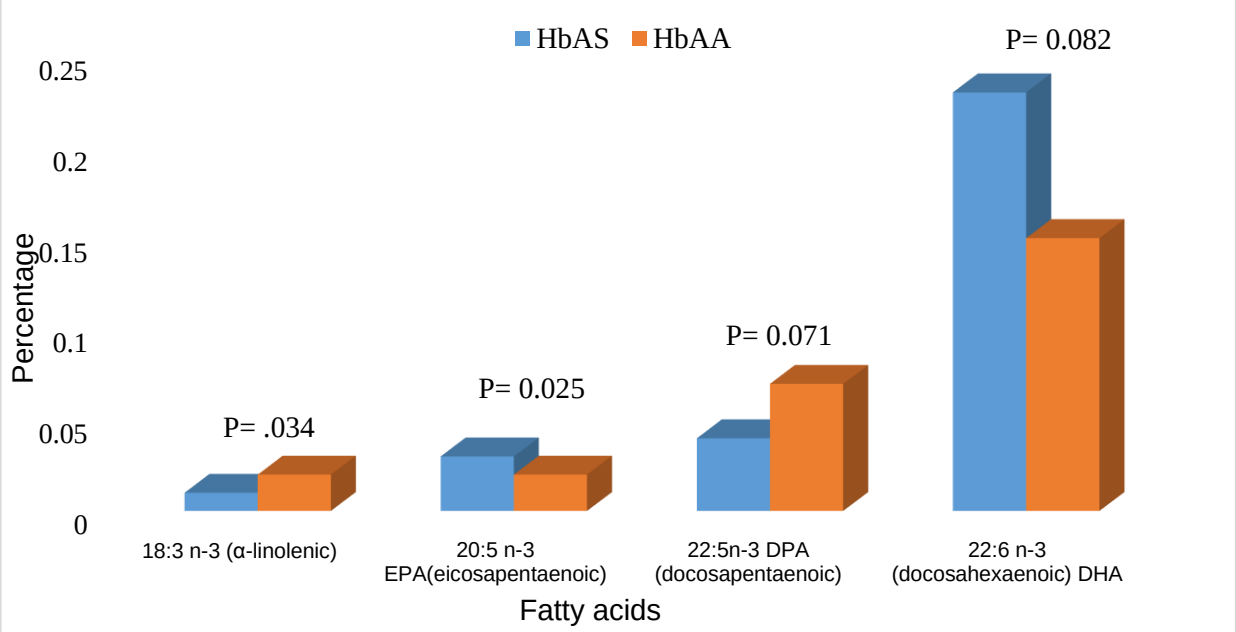


Figure 0-14 Levels of n3 fatty acids in RBCs of HbAS and HbAA women at delivery.

5.6 Discussion

The fatty acids that were found in the plasma and RBCs demonstrate that the two groups of women had different fatty acid profiles. These findings were not expected because the women were ethnically homogeneous and broadly comparable with regard to economic status (income), level of education, dietary background and daily food intake (number of daily meals).

Milk (cow, goat, camel) and milk products (homemade yoghurt, ghee and cheese), fava beans (broad beans), vegetables (mainly okra) and some meat (mutton, goat and camel) are consumed in Western Sudan. However, this region of the country is characterised by a high intake of carbohydrates from sorghum, millet and corn. This was reflected in the findings of high levels of palmitic (25.5% HbAS and 24.4% HbAA) and total saturated (33.6% HbAS and 32.0% HbAA) fatty acids in the plasma taken at baseline, and the palmitic (29.7% HbAS and 28.8% HbAA) and total saturated (52.4% HbAS and 43.8% HbAA) fatty acids that were found in the RBC analysis at the time of their babies' births (Figures 5.7 and 5.11).

The women with SCT had significantly higher levels of LA, AA and gamma-linolenic and total n6 fatty acids, and n6 metabolites, in their blood plasma at baseline compared with their healthy counterparts.

Similarly, they had higher levels of gamma-linolenic, adrenic and total n6 fatty acids, and n6 metabolites, in their RBCs at the time of delivery than the healthy control group. LA and AA levels in the RBCs were also higher in the SCT group than the healthy group, but did not reach statistical significance (Figures 5.9 and 5.13).

It is not known why the women with SCT had elevated levels of the n6 fatty acids compared with the control group. However, it may be linked to the lower income levels of the HbAS

group; they were not able to buy as much meat or fish as the healthy group, and, for some unknown other reasons, consumed more n6-rich oils, such as sesame, peanut and corn oils. Sesame and peanut oils are widely consumed in Western Sudan. The women in both groups had very low levels of n3 fatty acids. This is not a surprise, because preformed n3 fatty acids are rarely available in Western Sudan. Dry fish, which would be a potential source, is costly and not widely available, so people in this part of Sudan refrain from eating fish.

Generally, the diet of the western Sudanese contains a large amount of SFA, as they consume large amounts of carbohydrate. Acetyl coenzyme-A, which plays a large part in the breakdown of carbohydrate in the body, is available to metabolise essential fatty acids to their metabolites in the mitochondria.

In this study, we found that certain fatty acid levels in the healthy (HbAA) group of Sudanese pregnant women were lower than had been reported in pregnant women from Belgium during their first trimester; these fatty acids were c16:0 (palmitic), c18:0 (stearic) and the level of total SFA, and c20:4n6 (AA), c18:3n3 (α-linolenic), c22:6n3 (DHA) and the total amount of n3 acids (De Vriese, 2003). In fact, many previous studies showed higher levels of n3 fatty acids in pregnant women in other countries. The healthy pregnant Sudanese women had lower levels of EPA, DPA and DHA fatty acids in their RBCs than Norwegian pregnant women (0.02 vs. 0.23, 0.07 vs. 0.56 and 0.15 vs. 3.7 respectively) (Araujo *et al.*, 2020).

The level of DHA (C22:6n3) that was reported late in pregnancy among Chinese pregnant women was higher than that found in this study at delivery among Sudanese healthy pregnant women (2.54% vs. 0.15%) (Li *et al.*, 2015).

Levels of saturated, monounsaturated and n6 PUFAs usually increase during the period from early pregnancy until delivery. It is thought that this provides the fatty acids that the body

requires if adequate amounts are taken. At the same time, levels of AA and EPA tend to decrease during pregnancy, while DHA levels remain unchanged. This situation may put pregnant women at risk of low levels of essential fatty acids due to increased demand from the growing foetus (Aparicio, 2021).

Healthy women in Sudan have low levels of these essential fatty acids before they become pregnant, as their diet is not rich in fatty acids. After levels have fallen during pregnancy, the women breastfeed their new-borns, which means that their fatty acid levels are likely to drop further.

It has been reported that the breast milk of northern Sudanese women contains low levels of DHA (Nyuar *et al.,* 2013). It can be inferred from this finding that at the time of delivery, the fatty acid levels in the mothers' blood are low. Mothers who already have low levels of DHA, when exposed to prolonged lactation, will become more deficient in this fatty acid and may require supplements (Brenna *et al.,* 2007).

continuous supply of fish, eggs, meat, soybeans, sesame oil and other foods containing a diet rich in these essential fatty acids and stay healthy. We suggest that babies whose mothers have SCT should be observed and monitored for levels of essential fatty acids, as these may be low early in infancy.

There is increasing evidence of the beneficial role of DHA in growth. Interest in omega-3 (n3) fatty acids and their importance to health particularly during pregnancy is increasing. A study in China showed that the levels of fatty acids among Chinese women during pregnancy were not sufficient for healthy growth of the foetuses, and the researchers recommended that pregnant women should be offered more oily food that was rich in omega-3 fatty acids (Zhang *et al.,* 2009).

In this study, the sample size was low and levels of fatty acids at baseline in RBCs were not analysed (the samples were haemolysed during transport from Sudan to London). In addition, the fatty acid status of the babies at birth was not investigated. These limitations should be addressed in any future study. Regardless of the limitations, this study provides original data on fatty acid levels in women whose pregnancies are complicated by SCT.

Chapter 6

6. General discussion and conclusion

6.1 General discussion and conclusion

Sickle cell disease is an inherited genetic disorder of the blood. The inheritance is autosomal recessive. SCT is the heterozygote state of the disease, and SCA is the homozygote state which presents with severe illness and complications, of which the common concerns are vaso-occlusive crises, recurrent infections, chronic anaemia and organ damage. The majority of the body's systems are affected so the condition leads to growth abnormalities.

There are other causes of anaemia in this group, such as circumcision at age five leading to blood loss and also during the first day of wedding, and the sequence of perennial tears and repairs, as well as a third time during delivery for pregnant women.

The pathophysiology of the disease is thought to be due to a combination of different factors: genetic, cellular, hormonal and mechanical. The fatty acid composition of the cell membrane may play a part. The consanguinity of marriage, which is high in the area of study, plays a major role in the spread of the disease. Complications vary from patient to patient, depending on the haplotype, the region, and the severity of the attack. Other factors may worsen the patient's condition. The disease may be more serious or fatal during pregnancy.

Sickle cell disease, whether sickle cell anaemia, sickle cell trait or sickle cell associated with other blood disorders in Sudanese women has an adverse effect on general health. Before pregnancy, the health of the woman will be exposed to very frequent hospital admission and blood transfusion and her growth and development will be affected. During pregnancy, it will be more serious and may affect the progress of the pregnancy and the pregnancy outcome. This

study is important to investigate the status of this group of women whether childbearing age or pregnant.

In the previous studies, most of the investigators reported the prevalence of the disease in Sudan in different regions. No other studies showed the effect of the disease on the childbearing age group. No studies have been conducted in the region to examine the health of pregnant women with SCT or the outcome of their pregnancies. Fatty acids level in blood at early pregnancy and delivery were not measured in sickle cell traits in the previous studies.

So this study is new and original in the sub-Sahara area and Sudan. Involving only the pregnant sickle cell trait women. Because this group of women is usually looked after in the general clinic like normal healthy women without paying attention, they need special care early in pregnancy and more precautions late at the time of delivery.

Babies born to sickle cell trait women also need good care at the time of delivery and close follow-up thereafter. These babies need to be identified whether they are sickle cell anaemia, or sickle cell trait or normal. Most of the time, they are overlooked or missed until they present with symptoms at age 6 or 7 months. In the rural area the child will be given some local treatment and he will die without being diagnosed.

This study confirmed that the Cosnangeoutiy in marriage is more among those with sickle cell anaemia and sickle cell trait, than the control groups.

This study has revealed the nutritional and haematological status of Sudanese women of childbearing age who have SCA, as well as that of pregnant Sudanese women with SCT in comparison to an age-matched healthy control group. The results indicate that these two groups are at a disadvantage compared to their healthy peers due to their socio-economic background, consanguinity and other factors associated with poverty such as malnutrition and instability.

The levels of fatty acids in the blood were found to be lower than those reported from other countries; this suggests that people living in Western Sudan may not be getting sufficient essential fatty acids for optimal health. Moreover, there was evidence that pregnancy stress can worsen the condition of those with SCT.

Therefore, this study has provided evidence that pregnant women with SCT should be given tailored nutritional advice and multidisciplinary antenatal and postnatal care to improve their outcomes. It also suggests the need for a screening programme for SCD in all prenatal and postnatal clinics to identify those at risk of complications associated with pregnancy with SCT. The findings from this research will help the health policy makers, clinicians and public health professionals on interventions that are necessary to reduce the burden of SCD in Sudanese mothers and their babies.

To set up the research facilities for this study, many challenges were overcome; these included gathering funds, offering transportation, building communications and obtaining investigation tools and the reagents for the instruments. The following three studies showed clearly the importance of this research.

Study 1 (Chapter 3)

To see the effect of Sickle cell anaemia (SCA) on the general health of women, this study was conducted to investigate the nutritional and haematological status of Sudanese women of childbearing age who had (SCA). Anthropometry and haematology data were used to assess nutritional status and health and disease conditions, respectively.

Women with steady-state SCA (HbSS, n=39; average age =19.0 years) and without the condition (HbAA, n=36; average age 19.8 years) were recruited during a routine visit to the haematology clinic of the Ibn Auf Teaching Hospital in Khartoum, Sudan. The two groups of

women lived in similar environmental conditions and ate similar diets three times a day. However, despite taking regular meals, the women with SCA were thinner, lighter, and shorter than those who did not have the disease.

There was clear haematological variation between the two groups. The women with SCA showed low levels of haemoglobin, MCV and PCV. Some of these women took hydroxyurea but not regularly. There was no difference in the anthropometric and haematological variables between the women with SCA who were treated with hydroxyurea and those who were not.

The short height and low weight and BMI of the women with steady-state SCA compared with their healthy peers, and the abnormal haematological values that they showed, are a reflection of sustained nutritional insults that are inflicted by the disease.

Tailored nutritional counselling/advice must be an integral part of the management of patients with SCA. Such advice is vital, particularly for women, because of the adverse effects of pre-pregnancy nutritional deficiency on birth outcomes.

Parental relationship as 1st & 2nd degree relatives was high in the women with SCA. These women were educationally disadvantaged; very few of them had achieved higher education.

As we conducted this study, we noticed that girls who were diagnosed with SCA had less chance to marry. Many of those who were married to their cousins then underwent divorce soon after marriage because they had miscarried or could not conceive a child, due to the illness.

The study has provided solid evidence of the nutritional and health status of Sudanese women of childbearing age who have SCA. Their frequent need for hospital admission and blood transfusions, and low haemoglobin levels, are not compatible with pregnancy. Their external features and general build mean that they are overlooked when families seek wives for their boys.

6.2 Study 2 (Chapter 4)

This study aimed to investigate the haematological and anthropometric status of pregnant Sudanese women with SCT in comparison with an age-matched healthy control group.

The disease is caused by a single allele of the HbS gene, which produces abnormal β-chain haemoglobin. Carriers of SCT are usually asymptomatic, and they do not manifest the haematological or clinical abnormalities like that of SCA under normal conditions. However, there is evidence that they display some symptoms during stressful situations. The stress of pregnancy is physiological, and it is unclear whether or not this pregnancy stress negatively impacts pregnancy outcomes in women with SCT, particularly those from developing countries who struggle with nutritional deficiency.

Women with SCT (HbAS, n=34) and without (HbAA, n=60) were recruited during their first trimester. Detailed anthropometric, haematological, clinical, obstetric and birth outcome data were documented. Blood samples were collected at enrolment and at delivery of their baby.

A comparison of the haematological findings for the SCT and control groups in the first trimester showed no significant differences. However, at delivery, the women with SCT had lower levels of haemoglobin, PCV, MCH and neutrophil counts, and higher levels of MCV and PLT counts than the healthy women.

At birth, the babies of the women with SCT had lower birth weight and lower levels of haemoglobin, PCV and MCH, and higher counts of neutrophils and PLTs, than the babies born to healthy mothers. Moreover, there were more miscarriages, stillbirths, and admissions to the SCBU among the SCT group members than among the control group members.

Therefore, this study has revealed that SCT is associated with adverse pregnancy outcomes. Nevertheless, it is unclear whether the risk is restricted to undernourished women in Western

Sudan who have the condition or is universal to women with SCT. All women with SCT in Sudan should be regarded as at high risk, should they embark on pregnancy. Before they decide to have a child, they should be given pre-conceptual advice, and once they become pregnant, they should be given multidisciplinary antenatal and postnatal care.

A screening programme for SCD must be implemented in all prenatal clinics and all postnatal clinics for babies born to mothers with SCT. Those who are positive for the sickle cell gene should be categorised as high risk mothers. Screening should be carried out by experts, and the test method employed should be accurate and sensitive, such as haemoglobin electrophoresis, rather than a simple sickling solubility test, which can produce false-negative results.

6.3 Study 3 (Chapter 5)

This study aimed to compare the levels of fatty acids in the blood of pregnant women with SCT at baseline (first trimester) and at delivery with the same measures for healthy pregnant women.

At baseline, the levels of fatty acids that were found in the plasma demonstrated that the two groups of women had different fatty acid profiles. These findings were not expected because the women were ethnically homogeneous and broadly comparable with regard to economic status (income), level of education, dietary background and daily food intake (number of daily meals).

However, the people who live in this region of the country are characterised by a high intake of carbohydrates from sorghum, millet and corn. Indeed, this is reflected in the high levels of baseline palmitic and total SFAs that were found in the plasma of these women, and high levels at delivery of palmitic and total SFAs in the RBCs.

The women with sickle cell trait had significantly higher levels of LA, AA, gamma-linolenic acid, total n-6 fatty acids and n6 metabolites in their plasma. Similarly, they had higher levels

of gamma-linolenic and adrenic acid, total n6 fatty acids and n6 metabolites in their RBCs. LA and AA levels were also higher in their RBCs than was found for the healthy group, but the difference did not reach statistical significance. It is not clear why the women with SCT had elevated levels of n6 fatty acids compared with the control group. However, the HbAS group had slightly less income than the healthy group and perhaps bought less meat and fish, and, for unknown other reasons, consumed more n6-rich oils, such as sesame, peanut and corn. Sesame and peanut oils are widely consumed in Western Sudan. The women in both groups had very low levels of n3 fatty acids; this was not a surprise because preformed n3 fatty acids are rarely available in Western Sudan.

These Sudanese pregnant women had lower levels of fatty acids in their blood than women in China, Belgium, and the United States.

This study had some limitations, such as its small sample size and the inability to analyse the RBCs at baseline to discover their fatty acid content due to haemolysis. However, it is the first study of its kind in the region.

6.4 Conclusion

According to this research study, SCA negatively impacts the health of young girls and leads to them not being sought for marriage. Pregnant women with SCT should be treated as priority cases for attention. The screening of both mothers and their babies for SCD is recommended. Good nutrition and ingestion of fatty acid supplements are strongly recommended before conception and during pregnancy and lactation under the advice of a dietician. After diagnosis these women may need counselling and health educational advice.

6.5 Future studies

I propose that research should be conducted into:

- The role of omega-3 fatty acids in SCD in pregnancy;
- The appropriate proportions of the essential nutrients LA and linolenic acids that should be taken for best utilisation of both;
- Analysis of the levels of fatty acids at birth in the blood of babies born to mothers with SCT; and SCA.
- The effects of omega-3 supplements in pregnant Sudanese women with SCT.

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Appendix 1: Faculty of Medicine ethical approval

Faculty of Medicine
University of Khartoum
Ethics Research Committee
P. O. Box: 102
Khartoum, Sudan

كلية الطب
جامعة الخرطوم
لجنة أخلاقيات البحوث لمجمع العلوم الطبية
ص. ب : ١٠٢ الخرطوم - السودان

بسم الله الرحمن الرحيم

Ref: FM/DO/EC
Date: 10.2.2016

Ethical Clearance

On behalf of the ethics committee the following proposal was examined and approved for ethical clearance.

Pregnancy and birth outcomes and essential nutrient status of Sudanese pregnant women with sickle cell disease and sickle cell trait.

By:

Abdelrahim Osman Mohamed

UNIVERSITY OF KHARTOUM FACULTY OF MEDICINE
29 FEB 2016
DEAN'S OFFICE

Committee decision:

Approved	☑
Approved with clarifications	☐
Approved with modifications	☐
Not Approved	☐

S.A.Mahgoub
Prof. Sheikh Mahgoub
Head of Ethical Committee

S.K.Abass
Dr. Shaza Kamal Abass
Reporter

تحول الى لجنة اخلاقيات البحث
بوزارة الصحة.

Appendix 2: Ministry of Health

Republic of Sudan
Federal Ministry of health

Health Research council
National Health Research Ethics Review Committee

Date: 11/5/2016

Ethical Clearance Certificate

This is to certify that the proposal entitled ***(Pregnancy and birth outcomes and essential nutrient status of Sudanese pregnant women with sickle cell disease and sickle cell trait)*** *submitted by:* ***Dr. Eltigani Hassan Ali Margan*** *from* ***London Metropolitan University*** *has been approved by* ***the National Health Research Ethics Committee, Federal Ministry of Health*** *to be carried out in the Sudan.*

NB

The principal investigator is requested to submit the final report to the Research Directorate-Federal Ministry of Health.

15/5/2015

Dr Iman Abdalla Mustafa

Reporter Of the
National Health Research Ethics Review Committee

Appendix 3: Letter from El-Obeid Hospital

El-Obeid Obstetrics & Gynaecology Teaching HospitalDirector General Office

Dear Dr. Eltigani Hassan Ali Margan,

Principal Investigator,

Greetings,

I would like firstly to thank you for having our hospital as the centre for this important scientific research. It is quite well-known that scientific research is the driving force for the improvement of medical practice and since the proposed research and its results are of strong relevance to our missionand objectives we don't hesitate in giving our response of acceptance.

So there is no objection to conducting the research in El-Obeid obstetrics & Gynaecology Teaching Hospital as long as scientificand ethical procedures are considered.

Thanks, and wish you all success.

Dr. Ahmed Abdulkareem Ahmed .

Assistant Professor of Obstetrics & Gynaecology ,

Faculty of Medicine & Health Sciences University of Kordofan

Appendix 4: London Met Research Ethics Review Form

ELtigani ALi
Approved - 13/09/2016.

LONDON METROPOLITAN UNIVERSITY

LONDON MET RESEARCH ETHICS REVIEW FORM

For Research Students and Staff

Postgraduate research students (MPhil, PhD and Professional Doctorate): This form should be completed by all research students in full consultation with their supervisor. All research students must complete a research ethics review form before commencing the research or collecting any data and no later than six months after enrolment.

Staff: This form should be completed by the member of staff responsible for the research project (i.e. Principal Investigator and/or grant-holder) in full consultation with any co-investigators, research students and research staff before commencing the research or collecting any data.

Definition of Research
Research is to be understood as original investigation undertaken in order to gain knowledge and understanding. It includes work of direct relevance to the needs of commerce, industry, and to the public and voluntary sectors; scholarship*; the invention and generation of ideas, images, performances, artefacts including design, where these lead to new or substantially improved insights; and the use of existing knowledge in experimental development to produce new or substantially improved materials, devices, products and processes, including design and construction. It excludes routine testing and routine analysis of materials, components and processes such as for the maintenance of national standards, as distinct from the development of new analytical techniques. It also excludes the development of teaching materials that do not embody original research."

Scholarship is defined as the creation, development and maintenance of the intellectual infrastructure of subjects and disciplines, in forms such as dictionaries, scholarly editions, catalogues and contributions to major research databases."

London Met's *Research Ethics Policy and Procedures* and *Code of Good Research Practice*, along with links to research ethics online courses and guidance materials, can be found on the Research & Postgraduate Office Research Ethics webpage:
http://www.londonmet.ac.uk/research/current-students/research-ethics/

London Met's Research Framework can be found here:
http://www.londonmet.ac.uk/research/current-students/research-framework/

Researcher development sessions can be found here:
http://www.londonmet.ac.uk/research/current-students/researcher-development-programme/

This form requires the completion of the following three sections:

SECTION A: APPLICANT DETAILS
SECTION B: THE PROJECT - ETHICAL ISSUES
SECTION C: THE PROJECT - RISKS AND BENEFITS

SECTION A: APPLICANT DETAILS

A1	Background information
	Research project title: **Birth Outcomes and Essential Nutrient Status of Sudanese**

	Pregnant Women with Sickle Cell Disease and Sickle Cell Trait.
	Date of submission for ethics approval: **28/7/2016**
	Proposed start date for project: **October 2014 (Distance Learning)**
	Proposed end date for project: **October 2020**
	Ethics ID # (to be completed by RERP chair):

A2	**Applicant details, if for a research student project**
	Name: **Eltigani Hassan Ali**
	London Met Email address: **eha0029 @mylondonmet.ac.uk**

A3	**Principal Researcher/Lead Supervisor** Member of staff at London Metropolitan University who is responsible for the proposed research project either as Principal Investigator/grant-holder or, in the case of postgraduate research student projects, as Lead Supervisor
	Name: **: Professor Kebreab Ghebremeskel**
	Job title: **Lead Supervisor**
	London Met Email address: **: k.ghebremeskel@londonmet.ac.uk**

SECTION B: THE PROJECT - ETHICAL ISSUES

B1	**The Research Proposal**
	Please attach a brief summary of the research project including: ✓ The proposal ✓ The invitation letter to the participants . ✓ Ethical approval letter from Federal ministry of Health Sudan. ✓ Ethical approval letter from faculty of medicine University of Khartoum ✓ Letter from lead supervisor to the ethical committee Ministry of Health Sudan ✓ Letter of acceptance from hospital at area of study. ✓ The consent form.
B2	**Research Ethics** : proposal is attached with ethical consideration

	Please outline any ethical issues that might arise from this study and how they are to be addressed. ***NB** All research projects have ethical considerations. Please complete this section as fully as possible using the following pointers for guidance. Please include any additional information that you think would be helpful.* • Does the project involve potentially deceiving participants? **No** • Will you be requiring the disclosure of confidential or private information? **No** • Is the project likely to lead to the disclosure of illegal activity or incriminating information about participants? **No** • Does the project require a Disclosure and Barring Service (DBS) check for the researcher? **No** • Is the project likely to expose participants to distress of any nature? **No** • Will participants be rewarded for their involvement? **No** • Are there any potential conflicts of interest in this project? **No** • Are there any other potential concerns? **No** **If you answered yes to any of the points above, please explain.**
B3 **B4**	Does the proposed research project involve: • The analysis of existing data, artefacts or performances that are not already in the public domain (i.e. that are published, freely available or available by subscription)? **No** • The production and/or analysis of physical data (including computer code, physical entities and/or chemical materials) that might involve potential risks to humans, the researcher(s) or the University? **No** • The direct or indirect collection of new data from humans or animals? **Yes, Blood sample, about 5 ml, will be obtained from pregnancy women with and without sickle cell disease who consented to participate in the study.** • Sharing of data with other organisations? **Yes, co-investigators from the Faculty of Medicine, University of Khartoum, will have access to the generated data.** • Export of data outside the EU? **No** **If you answered yes to any of the points above, please explain.**
B5	Will the proposed research be conducted in any country outside the UK? If so, are there independent research ethics regulations and procedures that either: • Do not recognise research ethics review approval from UK-based research ethics services? **Yes, approval has been obtained from the Ethics Committee of:** 1. **Faculty of Medicine, University of Khartoum, Sudan** 2. **Federal Ministry of Health of Sudan** 3. **El Obeid Teaching Hospital , North Kordofan, Sudan (Study Area)** • Require more detailed applications for research ethics review than would ordinarily be conducted by the University's Research Ethics Review Panels and/or other UK-based research ethics services? **Yes, please see above** **If you answered yes to any of the points above, please explain.**
	Does the proposed research involve: • The collection and/or analysis of body tissues or fluids from humans or animals? **Yes** • The administration of any drug, food substance, placebo or invasive procedure to humans or animals? **No**

	• Any participants lacking capacity (as defined by the UK Mental Capacity Act 2005)? **No** • Relationships with any external statutory-, voluntary-, or commercial-sector organisation(s) that require(s) research ethics approval to be obtained from an external research ethics committee or the UK National Research Ethics Service (this includes research involving staff, clients, premises, facilities and data from the UK National Health Service (NHS), Social Care organisations and some other statutory public bodies within the UK)? **Yes, the relevant ethical approval has been obtained.** **If you answered yes to any of the points above, please contact your faculty's RERP chair for further guidance.**
B6	Does the proposed research involve: • Accessing / storing information (including information on the web) which promotes extremism or terrorism? **No** • Accessing / storing information which is security sensitive (e.g. for which a security clearance is required)? **No** **If you answered yes to any of the points above, please explain. To comply with the law, researchers seeking to use information in these categories must have appropriate protocols in place for the secure access and storage of material. For further guidance, see the Universities UK publication Oversight of Security Sensitive Research Material in UK Universities (2012).**

SECTION C: THE PROJECT - RISKS AND BENEFITS

C1	**Risk Assessment** Please outline: • the risks posed by this project to both researcher and research participants. **Please see blow** • the ways in which you intend to mitigate these risks. **Please see below** • the benefits of this project to the applicant, participants and any others. **Please see below** **Measures which will be undertaken to protect patients from risks and to maintain data confidentiality are outlined in the letter of the lead supervisor to the Ethics Committee of the Ministry of Health of Sudan and in the information leaflet for participants (attached).**

Please ensure that you have completed Sections A, B, and C and attached a Research Proposal before submitting to your Faculty Research Ethics Review Panel (RERP)

Please sign this form and submit it as an email attachment to the Chair of your faculty's Research Ethics Review Panel (RERP) and cc all of the staff and students who will be involved in the proposed research.

http://www.londonmet.ac.uk/research/current-students/research-ethics/

Research ethics approval can be granted for a maximum of 4 years or for the duration of the proposed research, whichever is shorter, on the condition that:

- The researcher must inform their faculty's Research Ethics Review Panel (RERP) of any changes to the proposed research that may alter the answers given to the questions in this form or any related research ethics applications
- The researcher must apply for an extension to their ethics approval if the research project continues beyond 4 years.

Declaration

I confirm that I have read London Met's *Research Ethics Policy and Procedures* and *Code of Good Research Practice* and have consulted relevant guidance on ethics in research.
Y

Researcher signature **Eltigani Hassan Ali (Student ID number: 07001067)**

Date : 28/7/2016

Feedback from Ethics Review Panel

	Approved	*Feedback where further work required*
Section A	Yes	
Section B	Yes	
Section C	Yes	
Date of approval		13.09.16
NB: The Researcher should be notified of decision within two weeks of the submission of the application. A copy should be sent to the Research and Postgraduate Office.		
Signature of RERP chair	D. McCarthy	

Appendix 5: Invitation letter

INVITATION TO PARTICIPATE IN NUTRITIONAL STUDY

Title of the study: Haematological and Nutritional status of Sudanese women with Sickle cell trait and anaemia. Does sickle cell trait compromise birth outcome?

Investigator: Eltigani Hassan Ali,

Lipidomics and Nutrition Research Centre, London Metropolitan University, London, United Kingdom

We are inviting you to take part in a nutritional study in pregnancy. The information given in this letter explains the purpose of the study. Please make sure you understand the purpose of the study before agreeing to participate. If you have any questions or require additional information please do not hesitate to ask (telephone number)

Background of the study: Sickle cell disease is a hereditary disease that affects a large number of people in Sudan and other countries. Most sickle cell patients suffer from blood, joint, lung bone, and other problems. Pregnant women with sickle cell disease and their developing babies are at greater risk of complications because of excessive vomiting and refusal of food which leads to loss of fluid and malnutrition.

Purpose of the Study: The purpose of the study is to find out the effect of the disease on the health and wellbeing of pregnant women with sickle cell disease and their children. The information obtained from this study will help us to improve how to take care of women with the disease before, during and after pregnancy.

The protocol of the study: We will ask a selected number of women with sickle cell trait and another group without the disease early in pregnancy. These will be followed until they give birth to a healthy baby. During this period, we will collect information about their diet, lifestyle and family circumstances, pregnancy and the treatment they received. In addition, we will collect about 5 ml of blood from them at the beginning and end of the study (recruitment and delivery). Women aged less than 16 years, mental disability and those who had a blood transfusion in the previous four months will not be studied.

Personal information and blood results will not be disclosed to anyone and will only be used for the purpose of this study.

Please note that you are free not to participate and may withdraw from the study at any time without giving any reason.

Contact telephone:

Date:

Appendix 6: Consent form

Consent form

Title of the study: Haematological and Nutritional status of Sudanese women with Sickle cell trait and anaemia. Does sickle cell trait compromise birth outcome?

I have read the attached information on the study in which I have been asked to participate and I have been given a copy to keep. I had the opportunity to discuss and ask questions about the study.

The investigator explained to me clearly the aim of the study. He made it clear to me that some relevant information about my nutrition, lifestyle and medical and obstetrical status will be collected. In addition, a small amount of blood, about 10 drops, will be taken from me at the beginning and end of my pregnancy. The aforementioned information and blood results will remain confidential and will only be used by the investigator for the purpose of the study.

I hereby fully and freely agree to participate in the study I understand that I can withdraw from the participation at any time without giving any reason.

Name of the patient: Hospital number: Patient's address: Telephone number:

As the investigator responsible for the study, I confirm that I have explained to the volunteer in detail the aims and benefits of the study and answered the questions asked.

Investigator's Name:

Signature

Contact telephone:

Date:

Appendix 7: Questionnaire pregnant 1

Haematological and Nutritional status of Sudanese women with Sickle cell trait and anaemia.

Does sickle cell trait compromise birth outcome?

Investigator: Eltigani Hassan Ali

Lipidomics and Nutrition Research Centre School of Human Sciences

London Metropolitan University, UK

Collaborator: Department of Biochemistry, Faculty of Medicine. University of Khartoum, Sudan

Demographic & Anthropometric Information	
Groups 1. Pregnant (AS). 2.Pregnant (AA)3.Non-pregnant (SS) 4. No pregnant normal (HbAA).	Q.1

Date of Enrolment (DD/MM/YY)		Q. 2
Name of Participant		Q. 3
Identification Number		Q. 4
Residence Address		Q. 6
Telephone Number		Q. 7
Other Contact Telephone		Q. 8
Age (years)		Q. 9
Weight (kg)		Q.10
Height (cm)		Q.11
Education of Participant	1. Illiterate 2. Primary School 3. Middle school 4. High school 5. University	Q.12
Education of Spouse	1. Illiterate 2. Primary School 3. Middle school 4. High school 5. University	Q. 13
Occupation of Participant	1. Employed 2. Self employed 3. House wife	Q. 14
Occupation of Spouse	1. Employed 2. Self employed 3. House wife	Q. 15
Household Income (Sudanese Pound)	1.< 1000 (Low income)	

	2.1000 -2000 (Average income) 3.>2000 (High income)	Q.16
House Ownership	1. Own house 2. Rent 3. Live with relatives	Q.17
Number of Family Members		Q.18
Number of siblings		Q.19
Birth order for the participant		Q.20
Parental Relationship in marriage	1. 1st degree Cousins 2. 2nd degree 3. None	Q.21
Tribe (participant)	1.Maseeria 2.Bedairia 3. Selehab 4. Masaleet 5. others ----------	Q.23
Tribe (spouse)	1.Maseeria 2.Bedairia 3. Selehab 4. Masaleet 5. others ----------	Q.24

Participant's medical History		
Age of participant at wedding		Q. 25
Years of marriage		Q.26
Age at first pregnancy (Years)		Q. 27
Number of living children		Q. 28
Number of sick children		Q. 29
Number of deceased children		Q. 30
Participant's Haemoglobin Test Result	1.Normal 2.Sickle Cell Trait 3.Sickle Cell Anaemia 4.Unknown	Q. 31
Years since Diagnosis		Q. 32
How often do you visit hospital for SCD?		Q. 33
Any Siblings with SCD	1. Yes… 2. No 3. Unknown	Q. 34
Any Children with SCD	1. Yes… 2. No 3. Unknown	Q. 35
Parental SCD Status	1. Yes … 2. No 3. Unknown	Q. 36
Spouse SCD status	1. Normal 2. Sickle Cell Trait	Q.37

	3.Sickle Cell Anaemia 4.Unknown	
Obstetric and birth outcome		
Did the participant tested for sickling	1. Yes 2. No	Q. 38
Hb electrophoresis. Done for type of Haemoglobin	1. Ye 2. No 3. unknown	Q. 39
Participant status at present	1. Normal 2. Sickle Cell Trait 3.Sickle Cell Anaemia 4.unknown	Q. 40
Preconception counselling (SCD)	1. Yes 2. NO	Q. 41
Vaccination (Pneumococcal)	1.Yes 2.No	Q. 42
Sickle cell crisis in (previous pregnancy)	1.Painful 2 Sequestration 3. Others 4. No	Q. 43
Sickle cell crisis in current pregnancy	1. Painful 2. Sequestration 3. Others 4. No	Q.44
Duration of hospital stay in the last admission	0 for no admission 1. any number	Q. 45
History of Blood transfusion	1.Yes 2. NO	Q. 46
Number of Blood Transfusion	0 no transfusion 1, any number	Q. 47
Current SCD Related Medication	a) Folic acid b) antibiotic c) Iron d) others ---------- e) No	Q. 48
Other Illnesses	(a) Diabetes b) Hypertension c) Others d) NO	Q. 49
Systolic BP (mmHg)		Q. 50
Diastolic BP (mmHg)		Q. 51
Gravid (Number of pregnancies)		Q. 52
Parity (Number of deliveries)		Q. 53
History of miscarriage		Q. 54

Gestational age at enrolment (weeks)		Q. 55
Attending Hospital/Clinic	1. Private 2. Government	Q, 56
Follow-up	1. Regular 2.Irregular	Q. 57
Mode of delivery this pregnancy	1. SVD 2. Induced 3.Forcep. 4. Vacuum 5. Caesarian	Q. 58
Gestational weeks at delivery		Q. 59
Gender of the baby	1. Male 2. Female	Q. 60
Birth weight		Q. 61
Head circumference at birth		Q. 62
APGAR SCORE	a. 1minute ( ) b. At 5 minutes ( )	Q. 63
SCBU admission	1. Yes 2. NO	Q. 64
Current deliver if still brith	1. Male 2. Female	Q. 65
Current Pregnancy Miscarriage	1. Yes 2. NO	Q. 66
Gestational age at miscarriage	In weeks	Q. 67
Physical Examination		
General condition	1. Good 2. Satisfactory 3. Poor	Q. 68
Head and Neck 0 normal 1. Text ……		Q. 69 a
Chest _____same as *above*_____		Q.69 b
CVS _____ same as above ____		Q.69 c
Abdomen _____ same as above _____		Q.69 d
Liver _____ same as above _____		Q.69 e
Spleen _____ same as above _____		Q.69 f
Kidneys _____ same as above _____		Q.69 g
CNS Haemiplegia, speech and epilepsy. 0 normal 1. Text……		Q.69 h

Others 0 normal 1. Text ……				Q69 I
Leg ulceration and deformities		1. Yes 2. No		Q. 70
Abdominal ultrasound		1. Yes 2. No		Q. 71
Urine protein		1. Positive 2. Negative		Q. 72
Blood collection, separation and processing				
Blood components	Enrolment	Term	Cord blood	Code
Haemoglobin	a.1	a.2	a.3	Q.73 a
PCV	b.1	b.2	b.3	Q.73 b
MCV	c.1	c.2	c.3	Q.73 c
MCH	d.2	d.2	d.3	Q.73 d
TWBC	e.1	e.2	e.3	Q.73 e
Neutrophils	f.1	f.2	f.3	Q.73 f
Lymphocytes	g.1	g.2	g.3	Q.73 g
platelets	h.1	h.2	h.3	Q.73 h
Sickling test	1 for +ve 2 for -ve			Q.73 I
Hb electrophoresis	1. AS 2. AA 3.SS			Q.73 J
RBS / mmol				Q. 74
Bilirubin	Not done			Q.75
Red blood cells	1. Yes 2. NO	1. Yes 2. NO	1. Yes 2.NO	Q.76
Plasma	1. Yes 2. NO	1. Yes 2. NO	1. Yes 2. NO	Q.77
Buffy coat	Not done	Not done	Not done	Q.78
Fatty acid profile	1. done	2.done	3. not done	Q79
Vitamin A	1.	2.	3.	Q80
Vitamin E	1.	2.	3.	Q81

Vitamin D	1.	2.	3.	Q82
B carotene	1.	2.	3.	Q83
Iodine	1.	2.	3.	Q84

Diet & Nutrition		
Question	Response	Code
Do you eat breakfast?	1. Yes 2. No	Q85
What do you usually eat?	a) Egg b) Cheese c) Milk d) Meat e) Fish f) Vegetable g) Bread h) Rice I) Fruits j)Others	Q86
Do you normally eat lunch?	1.Yes 2.No	Q87
What do you usually eat?	a) Egg b) Cheese c) Milk d) Meat e) Fish f) Vegetable g). Bread h) Rice I) Fruits j) Others	Q88
Do you normally eat dinner?	1. Yes 2. No	Q89
What do you normally have for dinner?	a) Egg b) Cheese c) Milk d) Meat e) Fish f) Vegetable g) Bread h) Rice I) Fruits j)Others	Q90
Do you eat with family members?	1. Yes 2. No	Q91
How many of you eat together?		Q92
Is there any food you do not eat during pregnancy?	1. Yes 2. No	Q93
Are you allergic to any food?	1. Yes ---------- 2. No	Q94
Do you suffer from morning sickness?	1.Yes 2.No	Q95
Have you gained weight during this pregnancy?	1.Yes 2.No	Q96

Appendix 8: Questionnaire non-pregnant 2

Haematological and Nutritional status of Sudanese women with Sickle cell trait and anaemia. Does sickle cell trait compromise birth outcome?

Investigator: Eltigani Hassan Ali

Lipidomics and Nutrition Research Centre School of Human Sciences

London Metropolitan University, UK

This Questionnaire designed for childbearing age non-pregnant women with their control

Groups 1. RED (AS). 2. Blue (AA) 3. Green non prgant (SS) 4. Black normal nonpregnant. Q.1		
Demographic & Anthropometric Information		
Question	Response	question
Date of Enrolment (DD/MM/YY)		Q2
Name of Participant		Q3
Identification Number		Q4
Residence Address		Q6
Telephone Number		Q7
Other Contact Telephone		Q8
Age (years)		Q9
Weight (kg)		Q10
Height (cm)		Q11
Education of Participant	1. Illiterate 2. Primary School 3. Middle School 4. High school 5. University	Q12
Household Income (Sudanese Pound)	1.< 1000 (Low income) 2.1000 -2000 (Average income) 3.>2000 (High income)	Q16
House Ownership	1. Own house 2. Rent	Q17

	3. Live with relatives	
Number of Family Members		Q18
Number of siblings		Q19
Birth order for the participant		Q20
Parental Relationship in marriage	1. 1st degree Cousins 2. 2nd degree 3. None	Q21
Tribe (participant)	1. Maseeria 2.Bedairia 3. Selehab 4. Masaleet 5. others ----------	Q23

Participant's medical History		
Question	Response	Code
Age of participant at first menstruation		Q25
Participant's Haemoglobin Test Result	1.Normal 2.Sickle Cell Trait 3.Sickle Cell Anaemia 4.Unknown	Q31
Years since Diagnosis		Q32
How often do you visit hospital for SCD?		Q33
Siblings with SCD	1. Yes… 2. No 3. unknown	Q34
Parental SCD Status	1. Yes … 2. No 3. Unknown	Q36
Did the participant test for sickling	1. Yes 2. No	Q38
Has the participant been tested for Hb electrophoresis?	1. Yes… 2. No 3. unknown	

Participant status	1. Normal 2. Sickle Cell Trait 3.Sickle Cell Anaemia 4.unknown	Q40
Has Participant given any counselling	1. Yes 2. NO	Q41
Vaccination pneumococcal	1.Yes 2.No	Q42
Sickle cell crisis	1. Painful 2 Sequestration 3. Others 4. No	Q43
Duration of hospital stay in the last	0 for no admission any number	Q45

admission		
History of Blood transfusion	1. Yes 2. NO	Q46
Number of Blood Transfusion	0 for no transfusion 1 any number	Q47
Current SCD Related Medication	a) Folic acid b) antibiotic c) Iron d) others ---------- e) No	Q48
Other Illnesses	(a) Diabetes b) Hypertension c) Others _____d) NO	Q49
Systolic BP (mmHg)		Q50
Diastolic BP (mmHg)		Q51
Attending Hospital/Clinic	1. Private 2. Government	Q56
Follow-up	1. Regular 2.Irregular	Q57
Physical Examination		
General condition	1. Good 2. Satisfactory 3.Poor	
Head and Neck 0 normal 1. Text ……		Q69 a
Chest _____same as *above*_____		Q69 b
CVS _____ same as above ____		Q69 c
Abdomen _____ same as above _____		Q69 d
Liver _____ same as above _____		Q69 e
Spleen _____ same as above _____		Q69 f
Kidneys _____ same as above _____		Q69 g
CNS Haemiplegia, speech and epilepsy. 0 normal 1. Text……		Q69 h
Others 0 normal 1. Text ……		Q69 I
Leg ulceration and deformities	1. Yes 2. No	Q70
Abdominal ultrasound	1. Yes 2. No	Q71

Blood collection, separation and processing				
Blood components	Enrolment			Code
Haemoglobin				Q73 a
PCV				Q73 b
MCV				Q73 c
MCH				Q73 d
TWBC				Q73 e
Neutrophils				Q73 f
Lymphocytes				Q73 g
platelets				Q73 h
RBS / mmol				Q74
Red blood cells	1. Yes 2. NO			Q76
Plasma	1. Yes 2. NO			Q77
Fatty acid profile				Q79
Vitamin A				Q80
Vitamin E				Q81
Vitamin D				Q82
B carotene				Q83
Iodine				Q84

Diet & Nutrition		
Question	Response	Code
Do you eat breakfast?	1. Yes 2. No	Q85
What do you usually eat?	b) Egg b) Cheese c) Milk e) Meat e) Fish f) Vegetable	Q86

	h) Bread h) Rice I) Fruits j)Others	
Do you normally eat lunch?	1.Yes 2.No	Q87
What do you usually eat?	a) Egg b) Cheese c) Milk e) Meat e) Fish f) Vegetable g). Bread h) Rice I) Fruits j) Others	Q88
Do you normally eat dinner?	1. Yes 2. No	Q89
What do you normally have for dinner?	a) Egg b) Cheese c) Milk d) Meat e) Fish f) Vegetable h) Bread h) Rice I) Fruits j)Others	Q90
Do you eat with family members?	1. Yes 2. No	Q91
How many of you eat together?		Q92
Is there any food you do not eat during pregnancy?	1. Yes 2. No	Q93
Are you allergic to any food?	1. Yes ---------- 2. No	Q94
Do you suffer from morning sickness?	1.Yes 2.No	Q95
Have you gained weight during this pregnancy?	1.Yes 2.No	Q96

Appendix 9: Follow-up card

Name of the participant .....Age.....

ID number .. (research number) .....

Address and phone.....

LMP ..... EDD .....

Gestational Age at delivery or Miscarriage .....weeks

Blood pressure RBS

Mode of delivery in the current pregnancy :

SVD	Induced V D.	Forceps	Vacuum extractor	CS

Fetal outcome :

Fresh Stillbirth	Macerated Stillbirth	Alive

If alive :

Newborn baby : Male..... Female.....Birth weight (kg).....

Head circumference .... cm . Body length in .....cm

Admission to Nursery YES ..... NO.....

APGAR score (1 minute).....(5 minutes).....

Name of Care Provider .....

Phone Number .....

Appendix 10: List of reagents, instrument and kits.

Solvents and Reagents:

Main Suppliers:

- Fisher Scientific (Bishop Meadow Road, Loughborough, LE11 5RG, UK).
- Sigma-Aldrich Company Ltd (The Old Brickyard, New Road, Gillingham, Dorset, SP8 4XT, UK).
- The BOC Group (The Priestley Centre, 10 Priestley Road, the Surrey Research Park, Guildford, Surrey, GU2 7XY, UK).
- Efamol Ltd (P.O. Box 51376. Waterfront. 8002. Cape Town, South Africa).

Product Name	Company	Catalogue No.
Solvents		
Methanol, 99.8%, HPLC-grade	Fisher Scientific	10124490
Chloroform, for HPLC	Fisher Scientific	10615492
Methanol, Extra Dry, for synthesis	Fisher Scientific	10468410
Petroleum Ether 60-80°C	Fisher Scientific	10448030
Heptane, ≈99% for HPLC	Fisher Scientific	10598800
Hexane	Fisher Scientific	10764371
Dichloromethane, 99+%, Extra Pure	Fisher Scientific	10458210
Ethanol, Absolute,		
Molecular Biology Grade	Fisher Scientific	10517694
Reagents		
Sodium sulfate, 99+%, granular, anhydrous	Fisher Scientific	10041501
Methylamine, extra pure,		
40 wt% solution in water		
Acetyl chloride, 98%	Fisher Scientific	10317520
Potassium bicarbonate, ≥99.5%	Sigma-Aldrich	60339
Sodium chloride, ≥99.5%	Sigma-Aldrich	S7653
Sodium sulfate, anhydrous, ≥99.0%	Sigma-Aldrich	71959
Butylated hydroxytoluene, ≥99%	Sigma-Aldrich	W218405
2',7'-Dichlorofluorescein	Fisher Scientific	10070080
Gas		
Oxygen-free Nitrogen the BOC group	SDS No:	000010021697

Laboratory Equipment

Main Suppliers:

- Merck Millipore (Suite 21, Building 6, Croxley Green Business Park, Watford, Hertfordshire, WD18 8YH, UK)
- Buchi (5 Whitegate Business Centre, Jardine Way, Chadderton, Oldham, OL9 9QL, UK)
- Techne (Cole-Parmer, Beacon Road, Stone, Staffordshire, ST15 OSA, UK)

- Thermo Fisher Scientific (Stafford House, 1 Boundary Park, Hemel Hempstead, HP2 7GE UK)
- UVItec Ltd (Avebury House, 36a Union Lane, Cambridge, CB4 1QB, UK)

Equipment Type	Company	Specification
Water Purifier	Merck Millipore Purite Neptune	(Serial No. 27655)
Rotary Evaporator	Buchi	Rota vapor R-210 Vacuum Pump V-700 Heating Bath B-491
Sample Concentrator	Techne	Dri-Block DB-3D
Ultra-Violet Lamp	UVItec	UVItec UV lamp (Serial No. 0613581)
Centrifuge	Thermo Fisher Scientific	Jouan C3i

Gas-Liquid Chromatograph

8000 series gas chromatograph model 8533 (HRGC MEGA 2 Series) with a flame ionisation detector (FID), split-split less injector and A200S autosampler.

Thermo Fisher Scientific (Stafford House, 1 Boundary Park, Hemel Hempstead, HP2 7GE UK)

i) Column

Product	BPX-70 Part No. 054612	
Specification	Length	30 m
	Type	Bonded phase
	Material	Fused silica
	Film Thickness	0.25 mm
	Internal Diameter	0.25 mm
	Operating temperature	min 50 ºC -max 260 ºC
	Conditioning temperature	260 ºC for 2 hours
Supplier	SGE	Analytical Science Europe Ltd. (1 Potters La, Milton Keynes MK113LA)

ii) Hydrogen Generator

Product	Brezza Hygen 400 Hydrogen Generator, Part No. CLB-H012
Flow rate	200 ml/min
Pressure	6 bar
Purity	99.9995%
Supplier	Jaytee Biosciences Ltd. (Altira Business Park, the Blvd, Herne Bay CT6 6GZ)

Fatty acids standard mix: 100 mg ampule contains the following with weight percentages Indicated

Trivial Name	Abbreviation	Weight %
Butyric acid Methyl Ester	4:0	4
Caproic acid Methyl Ester	6:0	4
Caprylic acid Methyl Ester	8:0	4
Undecanoic acid Methyl Ester	10:0	2
Lauric acid Methyl Ester	12:0	4
Tridecanoic acid Methyl Ester	13:0	2
Myristic acid Methyl Ester	14:0	4
Myristoleic acid Methyl Ester	14:1	2
Pentadecanoic acid Methyl Ester	15:0	2
Cis-10-Pentadecenoic acid Methyl Ester	15:1	2
Palmitic acid Methyl Ester	16:0	6
Palmitoleic acid Methyl Ester	16:1	2
Heptadecanoic acid Methyl Ester	17:0	2
Cis-10-Heptadecenoic acid Methyl Ester	17:1	4
Stearic acid Methyl Ester	18:0	4
Oleic acid Methyl Ester	18:1	4
Elaidic acid Methyl Ester	18:1	2
Linoleic acid Methyl Ester	18:2	2
Linolelaidic acid	18:2	2
α- linolenic acid Methyl Ester	18:3 2	2
γ- linolenic acid Methyl Ester	18:3	2
Arachidic acid Methyl Ester	20:0	4
Cis-11-eicosenoic acid Methyl Ester	20:1	2
Cis-11,14-eicosadienoic acid Methyl Ester	20:2	2

Cis-11,14,17-eicosatrienoic acid Methyl Ester	20:3	2
Arachidonic acid Methyl Ester	20:4	2
Cis-5,8,11,14,17-eicosapentaenoic acid Methyl Ester	20:5	2
Heneicosanoic acid Methyl Ester	21:0	2
Behenic acid Methyl Ester	22:0	4
Erucic acid Methyl Ester	22:1	2
Cis-13,16-docosadienoic acid Methyl Ester	22:2	2
Cis-4,7,10,13,16,19 docosahexaenoic acid Methyl Ester	22:6	2
Tricosanoic acid Methyl Ester	23:0	2
Lignoceric acid Methyl Ester	24:0	4
Nervonic acid Methyl Ester	24:1	2